CONFORMATIONALLY RESTRICTED POLYAMINE ANALOGS AS DISEASE THERAPIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority benefit of co-pending provisional patent application U.S. Serial No. 60/131,779, filed on April 30, 1999. The content of that application is hereby incorporated by reference herein in its entirety.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

Not applicable.

TECHNICAL FIELD

This invention relates to conformationally restricted polyamine analogs, and to porphyrin-polyamine conjugates. This invention also relates to uses of these analogs or conjugates in various medicinal applications, including suppressing cell proliferation and treating various diseases, including cancer.

BACKGROUND OF THE INVENTION

Natural polyamines, e.g., spermidine, norspermidine, homospermidine, 1,4-diaminobutane (putrescine), and spermine, are simple aliphatic amines produced in eukaryotic cells by a highly regulated metabolic apparatus. Polyamine levels and the

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activity of the polyamine biosynthetic apparatus tend to be high in dividing mammalian cells and low in quiescent cells. Populations of cells depleted of their polyamine content stop growing and may die. Janne et al. (1978) *A. Biochim. Biophys. Acta.* 473:241 and Pegg et al. (1982) *Am. J. Cell. Physiol.* 243:212-221. Polyamines are reviewed in Morgan (1998) *Methods. Mol. Biol.* 79:3-30.

Several lines of evidence indicate that polyamines, particularly spermidine, are required for cell proliferation: (i) they are found in greater amounts in growing than in non-growing tissues; (ii) prokaryotic and eukaryotic mutants deficient in polyamine biosynthesis are auxotrophic for polyamines; and (iii) inhibitors specific for polyamine biosynthesis also inhibit cell growth. Despite this evidence, the precise biological role of polyamines in cell proliferation is uncertain. It has been suggested that polyamines, by virtue of their charged nature under physiological conditions and their conformational flexibility, might serve to stabilize macromolecules, such as nucleic acids, by anion neutralization. Hafner et al. (1979) *J. Biol. Chem.* 254:12419; Pohjatipelto et al. (1981) *Nature* 293:475; Mamont et al. (1978) *Biochem. Biophys. Res. Commun.* 81:58; Bloomfield et al. (1981) in *Polyamines in Biology and Medicine*, Morris et al., Eds., Dekker, New York, pp. 183-205.

A treatment approach has been devised based on the observation that increases in the polyamine pool suppress polyamine biosynthesis. Porter et al. (1988) in *Advances in Enzyme Regulation*, Pergamon Press, pp. 57-79. This approach attempts to identify polyamine analogs which down-regulate polyamine biosynthesis, but which do not perform the polyamine functions required for cell growth. BESPM, a N-*bis*(ethyl) analog of spermine, has served as a model compound for this strategy. BESPM rapidly suppresses polyamine biosynthetic enzymes, depletes natural polyamine pools, and inhibits cell growth *in vitro*. Porter et al. (1987) *Cancer Res.* 47:2821-2825. In addition, BESPM suppresses polyamine uptake (Byers et al. (1990) *J. Physiol.* 142:460-467; and Kramer et al. (1993) *J. Cell. Physiol.* 115:399-407), and thus minimizes the ability of tumor cells to meet their polyamine requirement by taking them up from their environment. BESPM and related analogs also induce the polyamine metabolizing

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enzyme spermidine/spermine N^1-acetyltransferase (SSAT) in certain human carcinoma cell lines.

BESPM and other polyamine analogs have been used, or proposed for use, in treating a large variety of diseases, including a number of different cancers. U.S. Patent

5   No. 5,541,230. Polyamine analogs demonstrated, for example, potent antitumor activity against several melanoma cell lines and tumors *in vitro* (Porter et al. (1991) *Cancer Res.* 51:3715-3720; Shappell et al. (1992) *Anticancer Res.* 12:1083-1090) and *in vivo* using tumors growing as xenografts in athymic mice (Bernacki et al. (1992) *Cancer Res.* 52:2424-2430; Porter et al. (1993) *Cancer Res.* 53:581-586). Potent antitumor activity of

10   *bis*-ethyl spermine analogs has also been demonstrated for pancreatic cancer cell lines *in vitro* (Chang et al. (1992) *Cancer Chemother. Pharmacol.* 30:183-188) and *in vivo* (Chang et al. (1992) *Cancer Chemother. Pharmacol.* 30:179-182). Polyamine analogs have also been suggested for use in treating brain tumor therapy. Redgate et al. (1995) *J. Neurooncol.* 25:167-79. In addition to being useful against cancers of the brain,

15   pancreas, and skin, polyamine analogs are also useful against cancers of the bladder, bone, breast, colon, digestive tract, lung and ovaries. Chang et al. (1993) *J. Urol.* 150:1293-7; Snyder et al. (1994) *Anticancer Res.* 14:347-56; Yuan et al. (1994) *Biochem. Pharmacol.* 47:1587-92; Davidson et al. (1993) *Cancer Res.* 53:2071-5; Berchtold et al. (1998) *J. Cell. Physiol.* 174:380-6; Porter et al. (1988) *Adv. Exp. Med. Biol.* 250:677-90;

20   U.S. Patent Nos. 5,498,522 and 5,374,658. U.S. Patent No. 5,498,522 presents the use of spermidine/spermine N^1-acetyltransferase as a prognostic indicator of the efficacy of a polyamine analog against a malignant tumor.

Polyamine analogs have been used to treat cancer of the prostate. Mi et al. (1988) *Prostate* 34:51-60. Polyamines are produced in large amounts by the prostate gland and are abundant in the seminal fluid. Herr et al. (1984) *Cancer* 53:1294-8. Polyamine

25   analogs such as BE-4444, BE-373, and BE-333 are particularly effective in inhibiting prostate xenograft tumors in nude mice. Zagaja et al. (1998) *Cancer Chem. Pharm.* 41:505-512; Jeffers et al. (1997) *Cancer Chem. Pharm.* 40:172-179; Feuerstein et al. (1991) *J. Cell. Biochem.* 46:37-47; and Marton et al. (1995) *Ann. Rev. Pharm. Toxicol.*

30   35:55-91.

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In addition to treating cancer, polyamines and their analogs have uses in treating a number of other diseases and in numerous other medicinal applications. Oxidized polyamines are believed to inhibit growth of parasites (Morgan et al. (1983) *Adv. Polyamine Res.* 4: 169-174; Morgan et al. (1986) *Biochem. J.* 236:97-101; and U.S.

5   Patent No. 4,935,449) and suppress infectivity of selected strains of bacteria and fungi (Bachrach et al. (1971) *J. Gen. Virol.* 13:415-22; Nishimura et al. (1971) *Blochim. Biophys. Acta* 247:153-6; and U.S. Patent No. 5,744,453). Polyamines such as spermine and polyamine analogs are also anti-viral and some are anti-insecticidal. Bachrach et al. (1972) *Appl. Microbiol.* 23:232-5; Bachrach et al. (1971) *J. Gen. Virol.* 11:1-9; U.S.

10   Patent Nos. 5,021,409; 5,606,053; 5,608,061; 5,612,478; and 5,681,837. In addition, oxidized polyamines, such as spermine dialdehyde, for example, can be used in treatment of tissue grafts and other organs for transplantation. U.S. Patent No. 5,374,658. Polyamine analogs can also be used to treat neurodegenerative diseases and neurotrauma such as stroke. U.S. Patent Nos. 5,646,188 and 5,677,349. Polyamine analogs have also

15   been reported to be useful as anti-psoratic agents, and in the treatment of epilepsy, Alzheimer's disease, and multiple sclerosis, as described in U.S. Patent No. 5,646,188. Polyamine analogs are also useful in treating and preventing restenosis. U.S. Patent No. 5,516,807. Polyamine analogs are also useful in treatment of gastric ulcers. Igarashi et al. (1990) *Biochem. Biophys. Res. Commun.* 172:715-20. In addition, polyamine derivatives

20   including N-alkythio polyamine derivatives, polyamine thiols, and polyamine phenols are useful as radioprotective agents for normal tissues during radiotherapy. U.S. Patent Nos. 5,217,964; 5,354,782; and 5,434,145.

Polyamines and their analogs can be administered alone or in conjunction with additional agents. For example, therapeutic polyamines can be administered along with

25   1,3-*bis* (2-chloroethyl)-1-nitrosourea. U.S. Patent No. 5,541,230. In treating cancer, polyamines can be co-administered with various cytotoxic agents, including antineoplastic vinca alkaloids, antibiotics, antimetabolites, and platinum coordination complexes. U.S. Patent No. 5,654,287.

In addition to the various aforementioned medicinal uses, polyamines and

30   polyamine analogs have a variety of industrial uses, including derivativization of silica.

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U.S. Patent No. 5,763,388. Polyamines have also been used in conjunction with other clarification aids to treat wastewaters. U.S. Patent Nos. 5,413,719 and 5,707,532. The combination of aluminum chlorohydrate and a polyamine is an effective emulsion breaker for reverse (oil-in-water) emulsions, e.g. in a matrix comprising mostly oil as

5 encountered in a crude oil desalter unit. U.S. Patent No. 5,607,574. Polyamines are also useful in deodorizing polysulfides. U.S. S.I.R. H1,633. Polyamines are also used in industrial dyes. U.S. Patent No. 5,672,202. Polyamines and hot water can also be used in manufacturing microcapsules. U.S. Patent No. 5,401,443. The antioxidative and metal-chelating effects of polyamines are reviewed in Lóvaas (1997) *Adv. Pharmacol.* 38:119-

10 149.

It would be advantageous to develop novel polyamine analogs for various uses, including disease treatment.

All references cited herein are hereby incorporated by reference in their entirety.

15 SUMMARY OF THE INVENTION

The invention provides novel polyamine analogs, compositions comprising a polyamine analog, and methods using the analogs and compositions. In one embodiment, the polyamine analog is conformationally restricted.
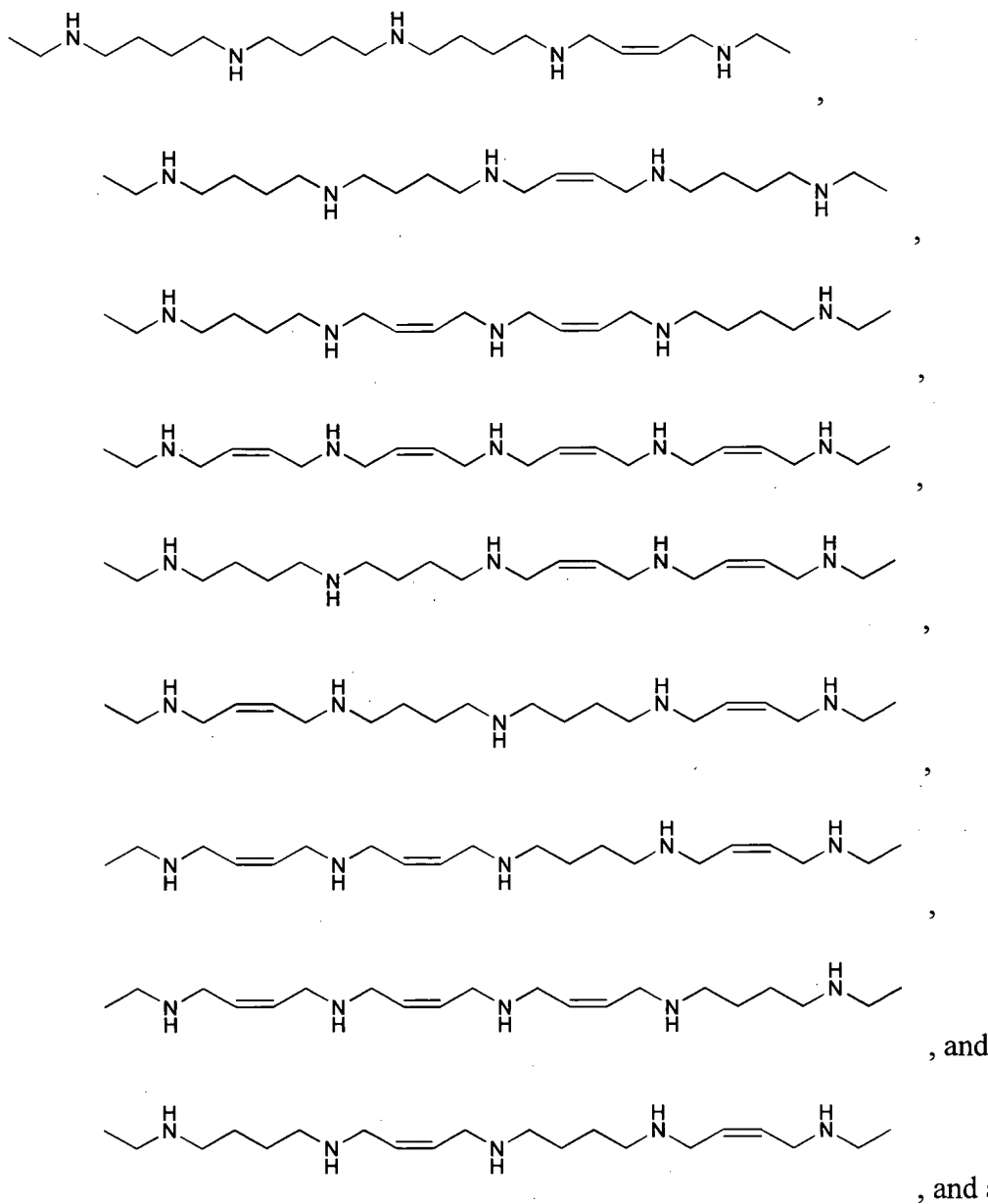
In another embodiment, the polyamine analog is selected from among compounds

20 of the formula:

E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-E

where A is independently selected from the group consisting of a single bond, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; B is independently selected from the group consisting of: a single bond,

25 C_1-C_6 alkyl, and C_2-C_6 alkenyl; and E is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; with the proviso that either at least one A moiety is selected from the group consisting of C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl, or at least one B moiety is selected from the group consisting of

C_2-C_6 alkenyl; and all salts and stereoisomers thereof. Specific embodiments of compounds of this type include

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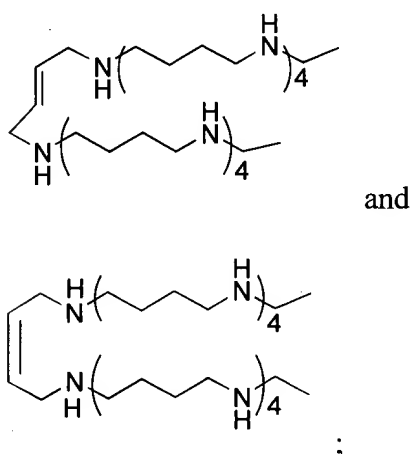, and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula:

15 E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH(-B-A-B-NH)$_x$-E

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wherein A is independently selected from the group consisting of a single bond, C_6-C_2 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; B is independently selected from the group consisting of a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; E is independently selected from the group consisting of

5 H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; and x is an integer from 2 to 16; with the proviso that either at least one A moiety is selected from the group consisting of C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl, or at least one B moiety is selected from the group consisting of C_2-C_6 alkenyl; and all salts and stereoisomers

10 thereof. Specific embodiments of compounds of this type include

and

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and all salts and stereoisomers thereof.

15 In another embodiment, the polyamine analog is selected from among the group of compounds of the formula

E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH(-B-A-B-NH)$_x$-E

wherein A is independently selected from the group consisting of a single bond, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl;

20 B is independently selected from the group consisting of a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; E is independently selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkanol, C_3-C_6 cycloalkanol, and C_3-C_6 hydroxyaryl, with the proviso that at least one E moiety be selected from the group consisting of C_1-C_6 alkanol, C_3-C_6 cycloalkanol,

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and C_3-C_6 hydroxyaryl; and x is an integer from 0 to 16; and all salts or stereoisomers thereof.

Specific embodiments of compounds of this type include

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and all salts and stereoisomers thereof.

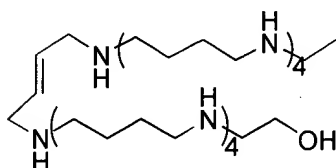In another embodiment, the polyamine analog is selected from among the group of compounds of the formula
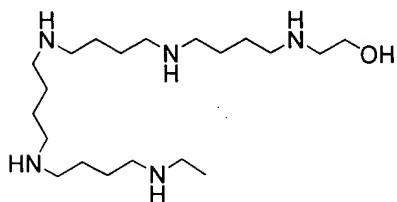
E-NH-D-NH-B-A-B-NH-D-NH-E

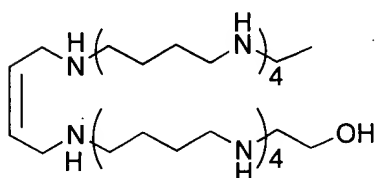wherein A is selected from the group consisting of C_2-C_6 alkynyl; B is independently selected from the group consisting of: a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; D is independently selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkenyl, and C_3-C_6 cycloaryl; and E is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; and all salts and stereoisomers thereof. Specific embodiments of compounds of this type include

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[Chemical structure: CH3CH2-NH-CH2CH2-NH-CH2-C≡C-CH2-NH-CH2CH2-NH-CH2CH3] ,

[Chemical structure: CH3-NH-CH2CH2-NH-CH2-C≡C-CH2-NH-CH2CH2-NH-CH3] , and

[Chemical structure: CH3CH2-N(H)-CH2CH2CH2CH2-NH-CH2-C≡C-CH2-NH-CH2CH2CH2CH2-N(H)-CH2CH3]

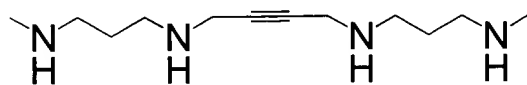5   ;

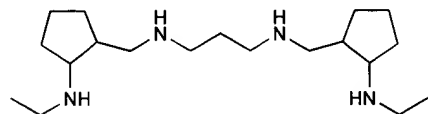and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula
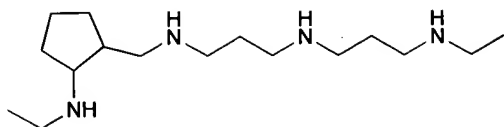
10   E-NH-B-A-B-NH-F-NH-B-A-B-NH-E

wherein F is selected from the group consisting of C_1-C_6 alkyl; A is independently selected from the group consisting of: a single bond, C_1-C_6 alkyl; C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; B is independently selected from the group consisting of: a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; and

15   E is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; with the proviso that either at least one A moiety is selected from the group consisting of C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl, or at least one B moiety is selected from the group consisting of C_2-C_6 alkenyl; and all salts

20   and stereoisomers thereof. Specific embodiments of compounds of this type include

[Chemical structure: bis(cyclopentyl) compound with ethyl-NH substituents] and

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and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group
of compounds of the formula

wherein A is independently selected from the group consisting of a single bond, C_1-C_6
alkyl; C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6
cycloalkenyl; B is independently selected from the group consisting of: a single bond,
C_1-C_6 alkyl, and C_2-C_6 alkenyl; with the proviso that A and both B moieties are not all a
single bond; and all salts and stereoisomers thereof. Specific embodiments of
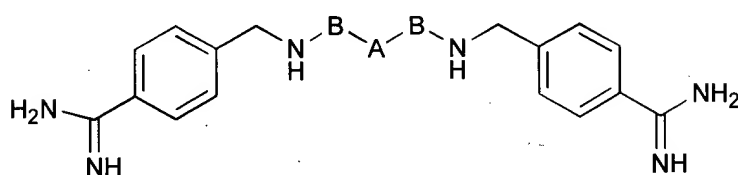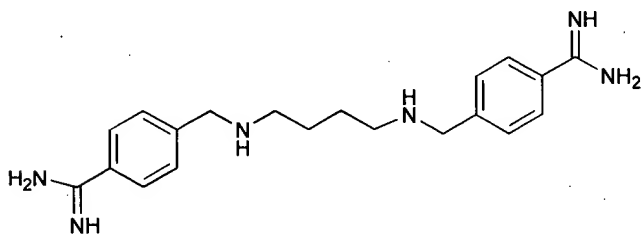compounds of this type include

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and all salts and stereoisomers thereof.

5   In another embodiment, the polyamine analog is selected from among the group

of compounds of the formula

E-NH-D-NH-B-A-B-NH-D-NH-E

wherein A is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6

alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkenyl, and C_3-C_6 cycloaryl; B is independently

10   selected from the group consisting of: a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; D is

independently selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6

alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkenyl, and C_3-C_6 cycloaryl; and E is

independently selected from the group consisting of H and C_1-C_6 alkyl; with the proviso

that either at least one A moiety is selected from the group consisting of C_2-C_6 alkenyl,

15   C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl, or at least one

B moiety is selected from the group consisting of C_2-C_6 alkenyl; and the proviso that at

least one E is selected from the group consisting of H or methyl; and all salts and

stereoisomers thereof. A specific embodiment of compounds of this type includes
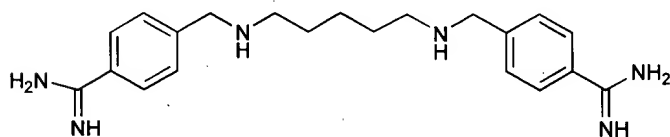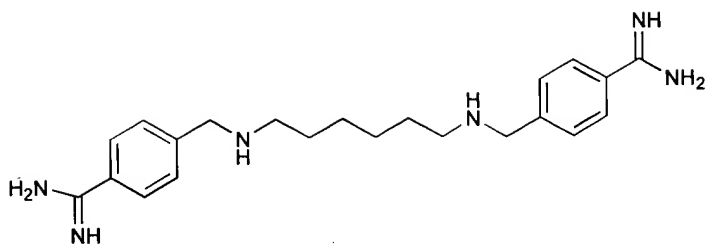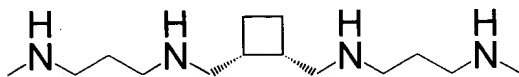

20   and all salts and stereoisomers thereof.

        In another embodiment, the polyamine analog is selected from among the group
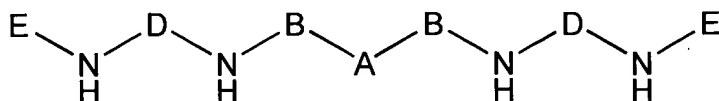
of compounds of the formula

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wherein A is *trans*-ethene (no stereochemical variation about the double bond being permitted, that is, *cis*-ethene is specifically excluded); B is independently selected from the group consisting of: a single bond, C_1-C_6 alkyl, and C_2-C_6 alkenyl; D is independently selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6

5   alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl, with the proviso that D is not C_3 alkyl; and E is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloaryl, and C_3-C_6 cycloalkenyl; and all salts thereof.

In another embodiment the invention also provides conjugates of polyamines to

10   porphyrin compounds, where the polyamines are linked via a covalent bond to the porphyrin compound. Polyamines useful for this embodiment of the invention include, but are not limited to, the polyamines described in the previous embodiments, or the polyamines depicted in Table 1. The covalent bond can be an amide bond, an amine bond, or any other suitable covalent bond. The polyamine analogs can be bound to the

15   porphyrin compound at positions including, but not limited to, any of the peripheral positions of the porphyrin macrocycle such as the β-pyrrole positions or the meso carbons of the macrocycle. Non-limiting examples of these compounds are given in Table 3.

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In one embodiment, the porphyrin-polyamine compounds are of the formula

where at least one of J_1-J_8 is independently selected from the group consisting of

-K_1-G-L-(N(P)-A)$_n$-K_2; where K_1 is independently selected from the group consisting of

5   C_1-C_8 alkyl and where the valence to the left of K_1 attaches to the porphyrin ring; where

G is -O-, -(C=O)-, -C(=O)-O-, -O-(C=O)-, -O-(C=O)-O-, -O-(C=O)-N-, -N-(C=O)-O-, or

a nonentity; where L is C_1-C_8 alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloaryl, C_1-C_8 alkoxy,

C_1-C_8 alkyl-C_3-C_8 cycloalkyl, C_1-C_8 alkyl-C_3-C_8 cycloaryl, C_1-C_8 alkoxy-C_3-C_8

cycloaryl, C_3-C_8 cycloalkyl-C_3-C_8 cycloaryl, C_3-C_8 cycloalkyl-C_1-C_8 alkyl, C_3-C_8

10   cycloaryl-C_1-C_8 alkyl, C_3-C_8 cycloaryl-C_1-C_8 alkoxy, C_3-C_8 cycloaryl-C_3-C_8 cycloalkyl,

or a nonentity; each A is independently selected from the group consisting of C_1-C_8 alkyl,

C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloaryl, C_3-C_8 cycloalkenyl, and

C_3-C_8 cycloalkynyl; P is selected from the group consisting of H and C_1-C_8 alkyl; n is an

integer from 2 to 8; and K_2 is independently selected from the group consisting of H,

15   C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloaryl, C_3-C_8

cycloalkenyl, C_3-C_8 cycloalkynyl, C_1-C_8 alkanol, C_3-C_8 cycloalkanol, and C_3-C_8

hydroxyaryl; where the remainder of the groups J_1-J_8 are each independently selected

from the group consisting of H, K_3, or K_4-COOH, where K_3 is independently selected

from the group consisting of C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 cycloalkyl,

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C_3-C_8 cycloaryl, C_3-C_8 cycloalkenyl, C_3-C_8 cycloalkynyl, C_1-C_8 alkanol, C_3-C_8 cycloalkanol, and C_3-C_8 hydroxyaryl; and K_4 is independently selected from the group consisting of C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloaryl, C_3-C_8 cycloalkenyl, and C_3-C_8 cycloalkynyl; and any salt or stereoisomer

5   thereof.

Additional embodiments include those where K_1 and each A are independently selected from the group consisting of C_1-C_8 alkyl, K_2 is independently selected from the group consisting of H and C_1-C_8 alkyl, and the remainder of the groups J_1-J_8 are each

10   independently selected from the group consisting of H, K_3, or K_4-COOH, where K_3 and K_4 are independently selected from the group consisting of C_1-C_8 alkyl. In additional embodiments, K_1 is -CH_2-CH_2- or -CH_2-CH_2-CH_2-. In additional embodiments, n is 4. In yet additional embodiments, J_3, J_4, J_7 and J_8 of the formula depicted above are independently selected from the group consisting of C_1-C_3 alkyl; J_5 and J_6 are

15   independently selected from the group consisting of C_1-C_3 alkyl and C_1-C_3 alkyl-COOH; and J_1 and J_2 are independently selected from the group consisting of
C_1-C_3 alkyl-G-N(P_1)-A-(NH-A)$_{n1}$-K_5;
where G is -(C=O)- or a nonentity; P_1 is H or C_1-C_3 alkyl; each A is independently selected from the group consisting of C_1-C_8 alkyl; n_1 is 3 or 4; and K_5 is independently

20   selected from the group consisting of H and C_1-C_8 alkyl.
The invention also includes methods of treating an indication in an individual comprising the step of administering to the individual a therapeutic amount of a porphyrin-polyamine conjugate of the invention.

The invention also provides methods of treating an indication comprising the step

25   of administering to the individual an effective amount of a composition comprising a polyamine analog, preferably a conformationally restricted polyamine analog.

In one method, the indication is cancer. In various embodiments, the cancer affects cells of the bladder, blood, brain, breast, colon, digestive tract, lung, ovaries, pancreas, prostate gland, or skin. In other embodiments, the indication can also include,

30   but is not limited to, Alzheimer's disease, epilepsy, multiple sclerosis, problems

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associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach ulcers, or tissue overgrowth after surgery. In other embodiments, the indication is an infection or infestation of parasites, bacteria, fungi or insects. The polyamine analog or porphyrin-polyamine conjugate is selected from the foregoing groups of compounds.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲),

10   SL-11128 (◊), SL-11129 (♦), SL-11130 (∇), SL-11133 (▼) on the survival of cultured human prostate cancer cells PC3.

ED_{50} of BE-4444 = 0.6 μM, SL-11121 = 0.52 μM, SL-11122 >31.25 μM,

SL-11123 >31.25 μM, SL-11126 = 0.2 μM SL-11127 >31.25 μM,

SL-11128 = 0.5 μM, SL-11129 = 1.7 μM, SL-11130 >31.25 μM, and

15   SL-11133 >31.25 μM.

Fig. 2 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲), SL-11128 (◊), SL-11129 (♦), SL-11130 (∇), and SL-11133 (▼) on the survival of cultured human prostate cancer cells DU145.

20   ED_{50} of BE-4444 = 0.07 μM, SL-11121 = 0.08 μM, SL-11122 = 0.08 μM,

SL-11123 = 0.51 μM, SL-11126 = 0.51 μM SL-11127 0.22 μM,

SL-11128 = 0.14 μM, SL-11129 = 0.32 μM, SL-11130 = 0.43 μM, and

SL-11133 = 0.34 μM.

Fig. 3 is a graph depicting the *in vitro* effect of increasing concentrations of BE-

25   4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲), SL-11128 (◊), SL-11129 (♦), SL-11130 (∇), and SL-11133 (▼) on the survival of cultured human prostate cancer cells DUPRO.

ED_{50} of BE-4444 = 0.2 μM, SL-11121 = 0.4 μM, SL-11122 = 0.56 μM,

SL-11123 >31.25 μM, SL-11126 = 1.1 μM, SL-11127 1.3 μM,

30   SL-11128 = 1.28 μM, SL-11129 >31.25 μM, SL-11130 >31.25 μM, and

15

SL-11133 = 31.25 μM.

Fig. 4 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 ( ),SL-11126 (Δ), SL-11128 (◊), on the survival of cultured human prostate cancer cells LNCAP.

5   ED_{50} of BE-4444 = 0.14 μM, SL-11121 = 0.14 μM, SL-11126 = 0.55 μM and

SL-11128 = 0.3 μM.

Fig. 5 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲), and SL-11128 (◊) on the survival of cultured human colon cancer cells HT29.

10   ED_{50} of BE-4444 = 0.5 μM, SL-11121 = 0.8 μM, SL-11122 = 0.8 μM,

SL-11123 =10.42 μM, SL-11126 = 1.5 μM, SL-11127 = 2.91 μM, and

SL-11128 = 1.35 μM.

Fig. 6 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), and SL-11126 (Δ) on the

15   survival of cultured human lung cancer cells A549.

ED_{50} of BE-4444 >31.25 μM, SL-11121 >31.25 μM, SL-11122 >31.25 μM,

SL-11123 >31.25 μM, and SL-11126 >31.25 μM.

Fig. 7 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), and SL-11126 (Δ) on the

20   survival of cultured human breast cancer cells MCF7.

ED_{50} of BE-4444 >31.25 μM, SL-11121 = 17.0 μM, SL-11122 >31.25 μM,

SL-11123 >31.25 μM, and SL-11126 = 0.7 μM.

Fig. 8 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (O), SL-11132 (■), and BE-333 (□) on the survival of cultured

25   human prostate cancer cells PC3.

ED_{50} of SL-11105 >31.25 μM, SL-11124 >31.25 μM, SL-11132 >31.25 μM

and BE-333 = 0.34 μM.

Fig. 9 is a graph depicting the *in vitro* effect of increasing concentrations of

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SL-11105 ( ), SL-11124 (O), SL-11132 (■), and BE-333 (□) on the survival of cultured human prostate cancer cells DU145.

ED_{50} of SL-11105 = 1.6 μM, SL-11124 >31.25 μM, SL-11132 = 0.015 μM and BE-333 = 0.12 μM.

5   **Fig. 10** is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (O), SL-11132 (■), and BE-333 (□) on the survival of cultured human prostate cancer cells DUPRO.

ED_{50} of SL-11105 = 0.43 μM, SL-11124 >31.25 μM, SL-11132 >31.25 μM and BE-333 = 0.9 μM.

10   **Fig. 11** is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (O), and BE-333 (□) on the survival of cultured human colon cancer cells HT29.

ED_{50} of SL-11105 = 25.2 μM, SL-11124 >31.25 μM, and BE-333 = 0.3 μM.

         Fig. 12 is a graph depicting the *in vitro* effect of increasing concentrations of

15   SL-11105 (●), SL-11124 (O), and BE-333 (□) on the survival of cultured human lung cancer cells A549.

ED_{50} of SL-11105 = 0.43 μM, SL-11124 >31.25 μM, and BE-333 = 0.3 μM.

         Fig. 13 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (O), and BE-333 (□) on the survival of cultured human breast

20   cancer cells MCF7.

ED_{50} of SL-11105 >31.25 μM, SL-11124 >31.25 μM, and BE-333 = 3.7 μM.

         Fig. 14 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●) and BE-333 (□) on the survival of cultured human brain tumor cells U251 MG NCI.

25   ED_{50} of SL-11105 = 25.9 μM, and BE-333 = 0.23 μM.

         Fig. 15A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human prostate cancer cells PC3.

ED_{50} of SL-11093 = 1.6 μM, SL-11098 = 1.4 μM, SL-11099 = 2.5 μM,

30   SL-11100 = 4.7 μM, SL-11101 = 7.7 μM, SL-11102 >31.25 μM and

BE-444 = 0.7 μM.

Fig. 15B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 ( ), SL-11104 (O), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (Δ), and BE-444 (♦) on the survival of cultured human prostate cancer cells PC3.

5   ED_{50} of SL-11103 >31.25 μM, SL-11104 >31.25 μM, SL-11108 = 2.2 μM,

SL-11114 = 0.7 μM, SL-11118 = 1.65 μM, SL-11119 >31.25 μM and

BE-444 = 0.7 μM

Fig. 16A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102

10   (Δ), and BE-444 (♦) on the survival of cultured human prostate cancer cells DU145.

ED_{50} of SL-11093 = 0.016 μM, SL-11098 = 0.02 μM, SL-11099 = 0.014 μM,

SL-11100 = 0.021 μM, SL-11101 = 0.22 μM, SL-11102 = 0.03 μM and

BE-444 = 0.03 μM.

Fig. 16B is a graph depicting the *in vitro* effect of increasing concentrations of

15   SL-11103 (●), SL-11104 (O), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119

(Δ), and BE-444 (♦) on the survival of cultured human prostate cancer cells DU145.

ED_{50} of SL-11103 = 2.8 μM, SL-11104 =9.4 μM, SL-11108 = 0.13 μM,

SL-11114 = 0.13 μM, SL-11118 = 0.05 μM, SL-11119 0.08 μM and

BE-444 = 0.03 μM.

20   Fig. 17A is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (♦) on the

survival of cultured human prostate cancer cells DUPRO.

ED_{50} of = SL-11099 = 0.08 μM, SL-11100 = 0.3 μM, SL-11101 = 0.85 μM,

SL-11102 = 0.15 μM and BE-444 = 0.2 μM.

25   Fig. 17B is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (Δ), and BE-444 (♦) on the

survival of cultured human prostate cancer cells DUPRO.

ED_{50} of SL-11108 = 0.98 μM, SL-11114 = 0.64 μM, SL-11118 = 0.25 μM,

SL-11119 = 0.44 μM and BE-444 = 0.2 μM.

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Fig. 18A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 ( ), SL-11098 (O), SL-11099 (■), SL-11100 (□), and BE-444 (◆) on the survival of cultured human prostate cancer cells LNCAP.

ED_{50} of SL-11093 = 0.21 μM, SL-11098 = 0.17 μM, SL-11099 = 0.21 μM,

5 SL-11100 = 0.7 μM, and BE-444 = 0.1 μM.

Fig. 18B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11108 (■), SL-11114 (□), SL-11118 (▲), and BE-444 (◆) on the survival of cultured human prostate cancer cells LNCAP.

ED_{50} of SL-11108 = 7.7 μM, SL-11114 = 3.0 μM, SL-11118 = 0.21 μM,

10 and BE-444 = 0.1 μM.

Fig. 19A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human colon cancer cells HT29.

ED_{50} of SL-11093 = 0.4 μM, SL-11098 = 0.4 μM, SL-11099 = 1.0 μM,

15 SL-11100 = 2.0 μM, SL-11101 = 5.2 μM. SL-11102 = 0.73 μM and

BE-444 = 0.93 μM.

Fig. 19B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (O), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (△), and BE-444 (◆) on the survival of cultured human colon cancer cells HT29.

20 ED_{50} of SL-11103 = 29.4 μM, SL-11104 = 25.8 μM, SL-11108 = 2.0 μM,

SL-11114 = 3.6 μM, SL-11118 = 0.98 μM, SL-11119 = 0.97 μM and

BE-444 = 0.93 μM.

Fig. 20A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human lung cancer cells A549.

25 ED_{50} of SL-11093 = 0.26 μM, SL-11098 = 0.29 μM, SL-11099 = 0.51 μM,

SL-11100 = 0.65 μM, SL-11101 = 2.2 μM, SL-11102 = 0.15 μM and

BE-444 = 0.15 μM.

Fig. 20B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 ( ), SL-11104 (O), SL-11108 (■), SL-11114 (□), SL-11118 (▲), and BE-444 (◆) on the survival of cultured human lung cancer cells A549.

ED_{50} of SL-11103 = 12.4 μM, SL-11104 >31.25 μM, SL-11108 >31.25 μM,

5   SL-11114 >31.25 μM, SL-11118 = 0.214 μM and BE-444 = 0.15 μM.

Fig. 21A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (◆) on the survival of cultured human breast cancer cells MCF7.

ED_{50} of SL-11093 = 0.66 μM, SL-11098 >31.25 μM, SL-11099 = 26.3 μM,

10   SL-11100 >31.25 μM, SL-11101 >31.25 μM SL-11102 >31.25 μM and

BE-444 >31.25 μM.

Fig. 21B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (O), SL-11108 (■), and BE-444 (◆) on the survival of cultured human breast cancer cells MCF7.

15   ED_{50} of SL-11103 >31.25 μM, SL-11104 >31.25 μM, SL-11108 >31.25 μM,

and BE-444 >31.25 μM.

Fig. 22A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (O), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (◆) on the survival of cultured human brain tumor cells U251 MG NCI.

20   ED_{50} of SL-11093 = 0.07 μM, SL-11098 = 0.1 μM, SL-11099 = 0.11 μM,

SL-11100 = 0.22 μM, SL-11101 = 1.7 μM, SL-11102 = 0.15 μM and

BE-444 = 0.2 μM.

Fig. 22B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (O), SL-11108 (■), and BE-444 (◆) on the survival of cultured

25   human brain tumor cells U251 MG NCI.

ED_{50} of SL-11103 = 9.5 μM, SL-11104 = 14.71 μM, SL-11108 = 2.0 μM,

and BE-444 = 0.2 μM.

Fig. 23 is a graph depicting the *in vitro* effect of increasing concentrations of

20

SL-11091 (   ), SL-11094 (O), and BE-343 (■) on the survival of cultured human prostate cancer cells PC3.

ED_{50} of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.24 μM.

Fig. 24 is a graph depicting the *in vitro* effect of increasing concentrations of

5   SL-11091 (●), SL-11094 (O), and BE-343 (■) on the survival of cultured human prostate cancer cells DU145.

ED_{50} of SL-11091 = 4.33 μM, SL-11094 = 15.4 μM, and BE-343 = 0.044 μM.

Fig.25 is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11091 (●), SL-11094 (O), and BE-343 (■) on the survival of cultured human colon

10   cancer cells HT29.

ED_{50} of SL-11091 >31.25 μM, SL-11094 = 28.8 μM, and BE-343 = 0.6 μM.

Fig. 26 is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11091 (●), SL-11094 (O), and BE-343 (■) on the survival of cultured human lung

cancer cells A549.

15   ED_{50} of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.2 μM.

Fig. 27 is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11091 (●), SL-11094 (O), and BE-343 (◆) on the survival of cultured human breast

cancer cells MCF7.

ED_{50} of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.5 μM.

20   **Fig. 28** is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11091 (●), SL-11094 (O), and BE-343 (■) on the survival of cultured human brain

tumor cells U251 MG NCI.

ED_{50} of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.14 μM.

Fig. 29 is a graph depicting the *in vitro* effect of increasing concentrations of

25   SL-11141(●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells PC3.

ED_{50} of SL-11141>31.25 μM, SL-11144 = 0.3 μM, and SL-11150 = 0.5 μM.

Fig. 30 is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11141 (   ), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate

30   cancer cells DU145.

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ED_{50} of SL-11141 = 0.13 μM, SL-11144 = 0.1 μM, and SL-11150 = 0.11 μM.

Fig. 31 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141 ( ), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells DUPRO.

5   ED_{50} of SL-11141 = 0.71 μM, SL-11144 = 0.36 μM, and SL-11150 = 0.48 μM.

Fig. 32 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141 (●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells LNCAP.

ED_{50} of SL-11141 = 0.07 μM, SL-11144 = 0.20 μM, and SL-11150 = 0.23 μM.

10   **Fig. 33** illustrates synthetic methodology used to prepare the compounds of the invention.

Fig. 34 illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 35 illustrates additional synthetic methodology used to prepare the

15   compounds of the invention.

Fig. 36 illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 37 illustrates additional synthetic methodology used to prepare the compounds of the invention.

20   **Fig. 38** illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 39 illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 40A illustrates additional synthetic methodology used to prepare the

25   compounds of the invention.

Fig. 40B illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 41 illustrates synthetic methodology used to prepare porphyrin-polyamine conjugages of the invention.

Fig. 42 illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugages of the invention.

Fig. 43 illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugages of the invention.

5   **Fig. 44** illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugages of the invention.

Fig. 45 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11162 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11162 are plotted in the X-axis and the corresponding surviving fractions of cells are

10   plotted in the Y-axis.

Fig. 46 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11184 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11184 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

15   **Fig. 47** is a graph depicting the *in vitro* effect of increasing concentrations of SL-11202 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11202 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 48 is a graph depicting the *in vitro* effect of increasing concentrations of SL-

20   11184 on the survival of human prostate cancer cells PC-3. The concentrations of SL-11184 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 49 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11202 on the survival of human prostate cancer cells PC-3. The concentrations of SL-

25   11202 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

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MODES FOR CARRYING OUT THE INVENTION

The present invention encompasses novel conformationally restricted polyamine analogs and compositions comprising these compounds. These analogs are useful as anti-proliferatives for suppressing cell proliferation. The analogs find use in treatment of various diseases, including use as anti-cancer agents in the treatment of various cancers. The analogs are also useful as anti-microbial agents. Novel polyamine analogs include the compounds shown in the synthetic schemes (Figures 33-40) and Table 1.

The invention also includes polyamine-porphryin conjugates. These conjugates include the compounds depicted in Table 2 and those shown in the synthetic schemes (Figures 41-44).

Definitions

By "polyamine analog" is meant an organic cation structurally similar but non-identical to polyamines such as spermine and/or spermidine and their precursor, diamine putrescine. By a "polyamine", a term well-understood in the art, is meant any of a group of aliphatic, straight-chain amines derived biosynthetically from amino acids; polyamines are reviewed in Marton et al. (1995) *Ann. Rev. Pharm. Toxicol.* 35:55-91. Polyamine analogs can be branched or un-branched. Polyamine analogs include, but are not limited to, BE-4444 [1,19-*bis* (ethylamino)-5,10,15-triazanonadecane]; BE-333 [N1,N11-diethylnorspermine; DENSPM; 1,11-*bis* (ethylamino)-4,8-diazaundecane; thermine; Warner-Parke-Davis]; BE-33 [N1,N7-*bis*(ethyl) norspermidine]; BE-34 [N1,N8-*bis*(ethyl) spermidine]; BE-44 [N1,N9-*bis*(ethyl) homospermidine]; BE-343 [N1,N12-*bis*(ethyl) spermine; diethylspermine-N1-N12; DESPM]; BE-373 [N,N'-*bis* (3-ethylamino) propyl)-1,7-heptane diamine, Merrell-Dow]; BE-444 [N1,N14-*bis*(ethyl) homospermine; diethylhomospermine-N1-N14]; BE-3443 [1,17-*bis*(ethylamino)-4,9,14-triazaheptadecane]; and BE-4334 [1,17-*bis*(ethylamino)-5,9,13-triazaheptadecane]; 1,12-Me$_2$-SPM [1,12-dimethylspermine]. Additional polyamine analogs are disclosed in International Patent Application WO 98/17624 and U.S. Patent No. 5,889,061. Various novel polyamine analogs are illustrated in the synthetic schemes in Figs. 33-40, and Table 1.

24

By "conformationally restricted" is meant that, in a polyamine analog, at least two amino groups in the molecule are locked or limited in spatial configuration relative to each other. The amino groups within the molecule may be primary, secondary, tertiary, or quartenary, and are preferably primary or secondary amino groups. The relative

5   movement of two amino groups can be restricted, for example, by incorporation of a cyclic or unsaturated moiety between them (exemplified, but not limited to, a ring, such as a three-carbon ring, four-carbon ring, five-carbon-ring, six-carbon ring, or a double or triple bond, such as a double or triple carbon bond). Groups restricting conformational flexibility by means of steric hindrance, yet structurally favorable to the anti-

10   proliferative, anti-cancer, or anti-microbial effects, can also be used according to the invention. A "conformationally restricted" polyamine analog can comprise at least two amino groups which are conformationally restricted relative to each other, but can also further comprise amino groups which are not conformationally restricted relative to each other. Flexible molecules such as spermine and BE-444 can have a myriad of

15   conformations and are therefore not conformationally restricted. Conformationally restricted polyamine analogs include, but are not limited to, the compounds listed in Table 1 and illustrated in Figures 33-40.

An "indication" includes any symptom or the like which points out a suitable remedy or treatment or which shows the presence of a disease or other unhealthful

20   condition. As used herein, an "indication" also includes a "disease" itself, where a disease is a condition of an organ, part, structure or system of the body in which there is incorrect function resulting from the effect(s) of heredity, infection, diet and/or environment, and/or other causes. The indication can include cancer. By "cancer" is meant the abnormal presence of cells which exhibit relatively autonomous growth, so that

25   they exhibit an aberrant growth phenotype characterized by a significant loss of cell proliferation control. Cancerous cells can be benign or malignant. In various embodiments, the cancer affects cells of the bladder, blood, brain, breast, colon, digestive tract, lung, ovaries, pancreas, prostate gland, or skin. In other embodiments, the indication can also include, but is not limited to, Alzheimer's disease, epilepsy, multiple

30   sclerosis, problems associated with tissue grafts and organ transplants, psoriasis,

25

restenosis, stomach ulcers, or tissue overgrowth after surgery. In other embodiments, the indication is an infection or infestation of parasites, bacteria, fungi or insects.

An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, rodents, primates,

5 and pets. Preferably, the individual is known or suspected to be afflicted by cancer or another disease treatable with a polyamine analog.

An "effective amount" or "therapeutic amount" is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of a polyamine

10 analog is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state. A therapeutic amount of a polyamine of the present invention is an amount sufficient to inhibit proliferation of diseased cells. A polyamine analog is considered to be an effective anti-tumor or anti-cancer agent if it is effective against at least one type of cancer cell line, even if it is not effective against a

15 different cell line.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization (i.e., not worsening) of state of disease, prevention of spread (i.e.,

20 metastasis) of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, improvement in quality of enjoyment of life, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

"Palliating" a disease means that the extent and/or undesirable clinical manifestations of a disease state are lessened and/or time course of the progression is

25 slowed or lengthened, as compared to not administering polyamine analogs of the present invention. Prefered polyamine analogs for methods of treating and palliating a disease include the compounds illustrated in Table 1.

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Polyamine analogs of the present invention

Novel polyamine analogs can be first designed based on current knowledge of polyamines and their analogs. Without wishing to be bound by any particular theory explaining polyamine analog toxicity, the inventors suggest that relevant knowledge

5   includes that related to polyamine interaction with DNA and ability to induce structural changes in nucleic acids. Feuerstein et al. (1991); Gosule et al. (1978) *J. Mol. Biol.* 121:311-326; Behe et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:1619-23; Jain et al. (1989) *Biochem.* 28:2360-2364; and Basu et al. (1990) *Biochem. J.* 269:329-334. Alternatively, a novel polyamine analog can be designed based on its likely ability to inhibit cell growth

10   by suppressing natural polyamine synthesis or depleting the intracellular natural polyamine pool. Porter et al. (1988) in *Advances in Enzyme Regulation*, Pergamon Press, pp. 57-79. Preferably, the novel polyamine analog is conformationally restricted. In the next step, the polyamine analog is tested *in vitro* for efficacy in inhibiting proliferation of diseased cells, such as tumor cells. If the polyamine analog passes this test, it can then be

15   tested in animals, such as nude mice with cancer xenografts. If the compound is found to be efficable, testing can then proceed to human trials.

The present invention encompasses novel polyamine analogs, such as those shown in Figs. 33-40 and Table 1. Polyamine analogs of the present invention are conformationally restricted. Conformation is a determinant of the spatial arrangement of

20   the pharmacophore or functional groups which interact with receptor binding sites. The latter prefer specific ligand conformations or a specific distribution of conformations. A flexible molecule such as spermine or BE-4444 can have a myriad of conformations. The conformer that binds to the macromolecule (e.g., DNA or RNA) may not necessarily be the one with the lowest energy as determined by spectroscopic methods or theoretically

25   by molecular mechanics calculations. The binding energy of the polyamine binding to the nucleic acid may be overcome with formation of an unstable conformer. Conversely, in the presence of a conformationally rigid analog of a flexible molecule, the host macromolecule might change its overall conformation or the distances from one strand to the other. Hydrogen bonding is the main binding force of either spermine or spermidine

30   associating with the helical region of a tRNA, and very likely also with DNA. Frydman et

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al. (1992) *Proc. Natl. Acad. Sci. USA* 89:9186-9191; and Fernandez et al. (1994) *Cell Mol. Biol.* 40: 933-944. The secondary amino groups present in the linear spermine analogs BE-343, BE-444, and BE-4444 are the groups most directly involved in the formation of the hydrogen bonds with the paired bases of tRNA. Therefore, these amino

5　groups that usually flank the central four-carbon or three-carbon segment of the polyamine analog can be considered as the pharmacophore of the polyamine analog. When the nitrogens are separated by only a two-carbon segment they are not protonated at pH 7.4 and hence they do not form hydrogen bonds. If these amino groups are locked into various configurations by the incorporation of cyclic or unsaturated moieties into the

10　polyamine analog molecule, a conformationally rigid analog is obtained. When such analogs bind to DNA or tRNA, they will very likely induce a change in the conformation of the nucleic acid strands or loops that may differ from the conformational changes induced by the natural polyamines. A series of conformationally restricted analogs of *bis*-ethyl-spermine that exhibited cytotoxic activities are illustrated in Table 1 and

15　described below.

Porphyrin-polyamine conjugates of the present invention

　　　The present invention also encompasses novel porphyrin-polyamine conjugates such as those depicted in Figs. 41-44 and in Table 3. These conjugates combine the

20　selective uptake of porphyrins by tumors with the cytotoxic and cytostatic effects of polyamine analogs. By "porphyrin-polyamine conjugate" is meant any porphyrin compound and any polyamine compound, linked by at least one, and preferably only one, covalent bond. Porphyrin-polyamine conjugates can incorporate any polyamine, whether that polyamine is conformationally restricted or not. Preferably, the polyamine conjugate

25　is conformationally restricted. By "porphyrin" is meant any compound incorporating the porphine ring or derivatives of the porphine ring. Preferred covalent linkages include amide linkages and amine linkages. Examples of porphyrin-polyamine conjugates are depicted in Table 2.

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Polyamine analogs and inhibition of cell growth

Polyamine analogs of the present invention are likely to be useful for treatment of a variety of diseases, including cancer, Alzheimer's disease, epilepsy, multiple sclerosis,

5   problems associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach ulcers, or tissue overgrowth after surgery, or an infection or infestation of parasites, bacteria, fungi or insects. In order to evaluate the efficacy of a particular novel polyamine for a particular medicinal application, the compounds can be first tested against appropriately chosen test cells *in vitro*. In a non-limiting example, polyamine analogs can be tested against tumor cells, for example, prostate tumor cells. On the basis

10   of the unique nature of polyamine metabolism in the prostate gland, exemplary experiments can utilize cell lines capable of growing in culture as well as *in vivo* in athymic nude mice, such as LNCaP. Horoszewicz et al. (1983) *Cancer Res.* 43:1809-1818. Culturing and treatment of carcinoma cell lines, cell cycle and apoptosis determinations based on flow cytometry; enzyme assays including ODC, SAMDC and

15   SSAT activities; and high pressure liquid chromatography detection and quantitation of natural polyamines and polyamine analogs are described in the art, for example, Mi et al. (1998) *Prostate* 34:51-60; Kramer et al. (1997) *Cancer Res.* 57:5521-27; and Kramer et al. (1995) *J. Biol. Chem.* 270:2124-2132. Evaluations can also be made of the effects of the novel polyamine analog on cell growth and polyamine-related metabolism. Analysis

20   begins with IC_{50} determinations based on dose-response curves ranging from 0.1 to 1000 μM performed at 72 hr. From these studies, conditions can be defined which produce about 50% growth inhibition and used to: (a) follow time-dependence of growth inhibition for up to 6 days, with particular attention to decreases in cell number, which may indicate drug-induced cell death; (b) characterize analog effects on cell cycle

25   progression and apoptosis using flow cytometry (analysis to be performed on attached and detached cells); (c) examine analog effects on polyamine metabolic parameters, including the biosynthetic enzymes ODC, SAMDC, the catabolic enzyme SSAT and polyamine pools themselves. Analog effects can be normalized to intracellular concentrations (by HPLC analysis), which also provide an indication of their relative

30   ability to penetrate cells. Marked differences in analog uptake can be further

characterized by studying analog ability to utilize and regulate the polyamine transporter, as assessed by competition studies using radiolabeled spermidine, as previously described in Mi et al. (1998).

5 In vivo *testing of polyamine analogs and porphyrin-polyamine conjugates*

Analogs and conjugates found to have potent or mechanism-based anti-proliferative activity *in vitro* towards cultured carcinoma cells can be evaluated in *in vivo* model systems. The first goal is to determine the relative toxicity of the analogs or conjugates in non-tumor-bearing animals, such as DBA/2 mice. Groups of three animals

10 each can be injected intraperitoneally with increasing concentrations of a compound, beginning at, for example, 10 mg/kg. Toxicity as indicated by morbidity is closely monitored over the first 24 hr. A well-characterized polyamine analog, such as BE-333, can be used as an internal standard in these studies, since a data base has already been established regarding acute toxicity via a single dose treatment relative to chronic toxicity

15 via a daily x 5 d schedule. Thus, in the case of new analogs, single dose toxicity relative to BE-333 is used to project the range of doses to be used on a daily x 5 d schedule.

After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor activity is determined. Typically, tumors can be subcutaneously implanted into nude athymic mice by trocar and allowed to reach 100-200 mm^3 before initiating treatment by

20 intraperitoneal injection daily x 5 d. Most analogs or conjugates can be given in a range between 10 and 200 mg/kg. Analogs or conjugates can be evaluated at three treatment dosages with 10-15 animals per group (a minimum of three from each can be used for pharmacodynamic studies, described below). Mice can be monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is determined by multi-

25 directional measurement from which volume in mm^3 is calculated. Tumors can be followed until median tumor volume of each group reaches 1500 mm^3 (i.e., 20% of body weight), at which time the animals can be sacrificed. Although the initial anti-tumor studies focuses on a daily x 5 d schedule, constant infusion can be performed via Alzet pump delivery for 5 days since this schedule dramatically improves the anti-tumor

30 activity of BE-333 against A549 human large cell hung carcinoma. Sharma et al. (1997)

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Clin. Cancer Res. 3:1239-1244. In addition to assessing anti-tumor activity, free analog levels or conjugate levels in tumor and normal tissues can be determined in test animals.

Methods of administration of polyamine analogs and porphyrin-polyamine conjugates

5   The polyamine analogs or porphyrin-polyamine conjugates of the present invention can be administered to an individual via any route known in the art, including, but not limited to, those disclosed herein. Preferably administration of the novel polyamine analogs or porphyrin-polyamine conjugates is by intravenous routes. Other methods of administration include but are not limited to, oral, intrarterial, intratumoral,

10   intramuscular, subcutaneous, intraperitoneal, gastrointestinal, and directly to a specific or affected organ. The novel polyamine analogs and porphyrin-polyamine conjugates described herein are administratable in the form of tablets, pills, powder mixtures, capsules, injectables, solutions, suppositories, emulsions, dispersions, food premixes, and in other suitable forms. Additional methods of administration are known in the art. The

15   pharmaceutical dosage form which contains the compounds described herein is conveniently admixed with a non-toxic pharmaceutical organic carrier or a non-toxic pharmaceutical inorganic carrier. Typical pharmaceutically-acceptable carriers include, for example, mannitol, urea, dextrans, lactose, potato and maize starches, magnesium stearate, talc, vegetable oils, polyalkylene glycols, ethyl cellulose,

20   poly(vinylpyrrolidone), calcium carbonate, ethyl oleate, isopropyl myristate, benzyl benzoate, sodium carbonate, gelatin, potassium carbonate, silicic acid, and other conventionally employed acceptable carriers. The pharmaceutical dosage form can also contain non-toxic auxiliary substances such as emulsifying, preserving, or wetting agents, and the like. A suitable carrier is one which does not cause an intolerable side effect, but

25   which allows the novel polyamine analogs or porphyrin-polyamine conjugates to retain its pharmacological activity in the body. Formulations for parenteral and nonparenteral drug delivery are known in the art and are set forth in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing (1990). Solid forms, such as tablets, capsules and powders, can be fabricated using conventional tableting and capsule-filling

30   machinery, which is well known in the art. Solid dosage forms can contain any number

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of additional non-active ingredients known to the art, including excipients, lubricants, dessicants, binders, colorants, disintegrating agents, dry flow modifiers, preservatives, and the like. Liquid forms for ingestion can be formulated using known liquid carriers, including aqueous and non-aqueous carriers, suspensions, oil-in-water and/or water-in-oil

5 emulsions, and the like. Liquid formulations can also contain any number of additional non-active ingredients, including colorants, fragrance, flavorings, viscosity modifiers, preservatives, stabilizers, and the like. For parenteral administration, novel polyamine analogs or porphyrin-polyamine conjugates can be administered as injectable dosages of a solution or suspension of the compound in a physiologically acceptable diluent or

10 sterile liquid carrier such as water or oil, with or without additional surfactants or adjuvants. An illustrative list of carrier oils would include animal and vegetable oils (peanut oil, soy bean oil), petroleum-derived oils (mineral oil), and synthetic oils. In general, for injectable unit doses, water, saline, aqueous dextrose and related sugar solutions, and ethanol and glycol solutions such as propylene glycol or polyethylene

15 glycol are preferred liquid carriers. The pharmaceutical unit dosage chosen is preferably fabricated and administered to provide a final concentration of drug at the point of contact with the cancer cell of from 1 μM to 10 mM. More preferred is a concentration of from 1 to 100 μM. As with all pharmaceuticals, the optimal effective concentration of novel polyamine analogs or porphyrin-polyamine conjugates will need to be determined

20 empirically and will depend on the type and severity of the disease, route of administration, disease progression and health and mass or body area of the patient. Such determinations are within the skill of one in the art. Polyamine analogs or porphyrin-polyamine conjugates can be administered as the sole active ingredient, or can be administered together, or in combination with another active ingredient, including, but

25 not limited to, cytotoxic agents, antibiotics, antimetabolites, nitrosourea, vinca alkaloids, polypeptides, antibodies, cytokines, etc.

The following examples are provided to illustrate but not limit the invention.

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EXAMPLES

Synthesis of conformationally-restricted polyamine analogs and of porphyrin-polyamine conjugates

5   a)   **Spermine and homospermine analogs containing a conformational restriction.**

Scheme 2 exemplifies a N^α, N^ω-bisethyl homospermine analog **7** containing a central *trans*-unsaturated bond. Amide **4** was prepared as described in Scheme 1 by alkylation of amide **1** with bromobutyronitrile to give **2**, followed by

10   reduction of the nitrile to the amine **3** that was mesitylsulfonated to **4**. Trans-allylic diester **5** was used to alkylate amide **4** and the tetramide **6** was obtained. Deprotection gave the *trans*-tetramide **7** (Scheme 2).

Introduction of a triple bond in the butane segment of homospermine also reduces its mobility. This was achieved by starting with the butyne diester **8** and following the

15   sequence of reactions outlined above (Scheme 3). Schemes 15-20 are further examples of the synthesis of polyamine spermine and homospermine analogs of this type.

b)   **Synthesis of pentamines with conformational restrictions.**

Schemes 4-14 are outlines of the syntheses of conformationally restricted pentamines. Scheme **4** depicts the reaction of *cis*-1-chloro-4-phthalimido butene with

20   amide **1** to give **11**. Hydrazinolysis of **11** gave **12** which was amidated to **13**. Reaction of the latter with 1,4-diiodobutane gave **14**, while reaction with equimolar amounts of *cis*-1,4-dichlorobutene gave **15**.

Amide **4** was alkylated with either 4-chlorobutyronitrile to give **16** or with *cis*-1,4-dichlorobutene to give **19**. Nitrile **16** was reduced with hydrogen over Ni Raney

25   to the amine **17** and the latter transformed in to the amide **18** (Scheme 5). Condensation of **18** with the chloroalkyl intermediate **15** gave the pentamide **20** that was deprotected to the pentamine **21** (Scheme 6). Condensation of **18** with the iodoalkyl derivative **14** gave **22** that was deprotected to the pentamine **23** (Scheme 7). Condensation of **18** and **19** gave pentamide **24** that was deprotected to the pentamine **25** (Scheme 8). Using **14** as the

30   alkylating agent, mesitylenesulfonamide was dialkylated to give **26**, and the latter

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deprotected to give **27** (Scheme 9). The analogous reaction carried out using **15** as alkylating agent, gave **28** and after deprotection led to the pentamine **29** (Scheme 10).

Alkylation of mesitylenesulfonamide with **19** gave the pentamide **30**, which was deprotected to **31** (Scheme 11). When **19** was used to alkylate an equimolar amount of mesitylenesulfonamide then **32** was obtained. Alkylation of **32** with **14** gave **33**, that was deprotected to give **34** (Scheme 12). When the chloroalkyl intermediate **15** was used to alkylate one equivalent of mesitylenesulfonamide, then the triamide **35** was obtained. Reaction of **35** with **14** gave **36** which was then deprotected to **37** (Scheme 13). Condensation of **35** and **19** gave the pentamide **38** that was deprotected to **39** (Scheme 14). The above mentioned Schemes describe the synthesis of *cis*-compounds. The same synthetic methodology can be used to obtain the *trans*-isomers, or *cis* and *trans* bonds in different segments within the same molecule.

c)   **Polyamine analogs with diamidine substituents.**

A new class of polyamine analogs is shown in Scheme 21. They derive from 1,4-dibenzylputrescine, 1,5-dibenzylcadaverine, and 1,6-dibenzylhexanediamine. They are diamidine derivatives, where the diamidine residues are carrier groups that have been shown to be efficient in the transport of drugs into different protozoa. The general procedure of synthessis was based on the condensation of 4-cyanobenzaldehyde with the diaminoalkanes to give the Schiff bases, followed by reduction *in situ* to the corresponding dinitriles **68**. The latter were converted to the diamidines **69** through their iminoethers.

d)   **Synthesis of oligoamines.**

Scheme 22 describes the synthesis of a N-2 hydroxyethyl derivative of a pentamine such as **75**. Starting wtih **18**, alkylation with 4-bromobutyronitrile gave **70**. Reduction of the nitrile of **70** and mesitylenesulfonylation of the resulting amino group gave **71**. It was alkylated again with 4-bromobutyronitrile to give **72**, and again reduced and mesitylsulfonylated to give **73**. The latter was then alkylated with the benzyl ester of

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2-bromoethanol to give **74**. Treatment with hydrobromic acid in acetic acid cleaved both the mesitylene sulfonyl protecting groups and the benzyl ether residue to give **75**.

Scheme 23 reports the synthesis of a *trans*-decamine **77** and of a *cis*-decamine **79**. Starting with the pentamide **73** (Scheme 22) and by reaction with *trans*-diester **5** (Scheme 2) the decamide **76** was prepared, which on deprotection gave **77** as a decahydrochloride. In an analogous manner, by condensation of **73** with the *cis*-1,4-dimesityleneoxy-2-butene, the decamide **78** was prepared, which on deprotection gave **79** as a decahydrochloride.

Scheme 24 outlines the synthesis of a N-2 hydroxyethyl *trans*-decamine **92** and a *cis*-2-hydroxyethyl decamine **95**. The procedure repeats almost all the procedures described in the foregoing schemes. The synthesis of **80** proceeded by alkylating BOC-mesitylenesulfonamide with the benzyl ester of 2-bromoethanol. Cleavage of the BOC protecting group leads to **81**, alkylation with 4-bromobutyronitrile then gave **82**, and after reduction of the nitrile group and reaction with mesitylene sulfonyl chloride the diamide **83** was obtained. Again, alkylation with 4-bromobutyronitrile led to **84**, reduction and mesitylsulfonylation gave **85**, alkylation of **85** gave **86**, reduction and mesitylsulfonylation gave **87**, and alkylation, reduction and mesitylsulfonylation performed on **87** gave **89**. Alkylation of **73** with *trans*-1,4-dibromo-2-butene gave **90**. Alkylation of **89** with **90** gave **91**, which after deprotection gave the *trans*-ω-hydroxy-decamine **92**. Alkylation of **73** with *cis*-1,4-dichloro-2-butene gave **93**. Alkylation of **89** with **93** gave **94**. Deprotection of **94** gave the *cis*-ω-hydroxy-decamine **95**, isomeric with **92**.

e)   **Synthesis of porphyrin-polyamine conjugates.**

Scheme 25 outlines the synthesis of porphyrin-polyamine. Starting with the above mentioned amide 18 (Scheme 5), alkylation with the benzyl ether of 4-bromobutanol gave **96**. Treatment with acid cleaved both the sulfonate and the benzyl protecting groups to give **97**. Protection of the free amino residues with Boc followed by Mitsunobu reaction using phthalimide gave **99**. Cleavage of the phthalyl protecting group with

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hydrazine hydrate gave polyamine **100**. It was condensed with mesoporphyrin IX to give **101**, and after cleavage of the Boc protecting group **102** (SL-11162) was obtained.

Scheme 26 outlines the synthesis of a porphyrin-bisethylpolyamine conjugate. The synthesis of the intermediate **105** follows the pattern described above. The primary

5 alcohol is oxidized to the aldehyde **106** in Swern oxidation reaction. The aldehyde **106** was then condensed with ethylamine using a reductive alkylation reaction that gave **107**. The latter was coupled with mesoporphyrin IX using amide forming procedures and the resulting **108** was deprotected in acid to **109** (SL-11184).

Scheme 27 illustrates on the preparation of a porphyrin-polyamine conjugate,

10 where an amine group tethers the polyamine to the porphyrin. The amide derivative **111** was prepared as described above and was then reduced using metal hydrides and deprotected to yield **112.** The synthesis of the porphyrin-polyamine **112** could also be achieved as shown in the Scheme 28. Reduction of the diester of mesoporphyrin IX under controlled conditions gave the dialdehyde **113**. Reductive alkylation of **100** with **113**

15 followed by deprotection also gave **112** (SL-11202).

EXAMPLE 1

Synthesis Of Polyamine Compounds

Compound 2: NaH (80%, 1.08 g, 36 mmol) was added to a solution of amide **1** (6.81 g, 30 mmol) in DMF (50 ml) in an ice-water bath under N_2. The mixture was stirred for 1 h

5 and a solution of 4-bromobutyronitrile (4.88 g, 33 mmol) in DMF (10 ml) was added in portions. The mixture was stirred over night at 75°C. The solvent was distilled off, the residue taken up in chloroform washed with a saturated solution of ammonium chloride, dried (Na_2SO_4) and evaporated. The residue was purifid by flash chromatography on silica gel (hexane/ethyl acetate 3:1) to yield 8.0 g (90%) of **2** as a colorless oil. ^{1}H-NMR

10 (CDCl$_3$) δ 1.05 (t, 3H), 1.90 (m, 2H), 2.30 (b, m, 5H), 2.60 (s, 6H), 3.20 (q, 2H), 3.35 (t, 2H), 6.95 (s, 2H); ^{13}C-NMR (CDCl$_3$): δ 12.50, 20.61, 22.43, 23.60, 31.05, 36.12, 40.39, 43.78, 118.62, 131.79, 132.67, 139.71, 142.41. MS-EI (m/z) 294 (M^+).

Compound 4: Nitrile **2** (7.8 g, 27 mmol) was dissolved in a mixture of ethanol (150 ml) and concentrated hydrochloric acid (1.5 ml). PtO_2 was added (700 mg) and the mixture

15 was hydrogenated at 50 psi over night. The catalyst was filtered off and the solvent evaporated. The residue (78 g, 98%) was used in the next step without further purification. The free base gave ^{1}H-NMR (CDCl$_3$) δ 1.00 (t, 3H), 1.55 (m, 4H), 2.25 (s, 3H), 2.80 (t, 2H), 3.20 (m, 4H), 6.95 (s, 2H); ^{13}C-NMR (CDCl$_3$): δ 12.54, 20.69, 22.53, 24.72, 27.65, 39.92, 40.29, 44.59, 131.71, 133.21, 139.82, 142.09. FAB-MS (m/z) 299

20 ($M^+ + 1$). Mesitylenesulfonyl chloride (8.8 g, 40.5 mmol) in dioxane (30 ml) was added dropwise to a stirred mixture of compound **3** (7.8 g, 27 mmol) dissolved in dioxane (60 ml) and 50% KOH (30 ml) at 0°C. The reaction mixture was allowed to reach 20°C and then kept over night. An excess of water was added and the mixture was extracted with chloroform, dried (Na_2SO_4) and evaporated. The oily residue was crystallized from ethyl

25 acetate/hexane yielding **4**; 10.9 g (82%); mp 71.5-72°C. ^{1}H-NMR (CDCl$_3$) δ 1.00 (t, 3H), 1.10-1.50 (m, 4H), 2.30 (s, 6H), 2.55, 2.60 (s, 12H), 2.85 (q, 2H), 3.15 (m, 4H), 4.70 (t, 1H), 6.95, 7.00 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 12.70, 20.92, 21.04, 22.73, 22.92, 24.58, 26.68, 40.04, 42.02, 44.42, 131.91, 133.31, 133.64, 138.99, 140.05, 142.15, 142.35. MS-FAB (m/z) 480 (M^+).

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Compound 5: (E)-2-Butene-1,4-diyl bis[mesitylenesulfonate] : (E)-2-Butene-1,4-diol (1.76 g, 20 mmol), and benzyltriethylammonium bromide (270 mg, 1 mmol) were dissolved in 30 ml of a 50% potassium hydroxide solution and 30 ml of dioxane. The mixture was stirred at 5°C and mesitylenesulfonyl chloride (8.72 g, 40 mmol) dissolved

5   in 30 ml of dioxane was added dropwise. When the addition was over, stirring was continued for 1 h, water was then added, and the white precipitate was filtered and crystallized from chloroform-hexane to yield **5**; 7.0 g (77%); mp 119-120°C. ^{1}H-NMR (CDCl^3): δ 2.35 (s, 6H), 2.60 (s, 12H), 4.45 (d, 4H), 5.75 (b, 2H), 6.95 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 20.96, 22.52, 67.96, 127.67, 131.69, 131.74, 139.79, 143.45. MS-EI (m/z),

10   452 (M$^+$), 253, 200, 183. Anal. Calcd for C$_{22}$H$_{28}$O$_6$S$_2$: C, 58.40; H, 6.19. Found: C, 58.35; H, 6.22.

Compound 6 was synthesized from **5** according to a procedure described elsewhere (Reddy et al., *J. Med. Chem.* 41:4723 (1998)) in 56% yield . ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, J=7.15 Hz, 6H, CH$_3$), 1.34 (m, 8H, CH$_2$), 2.29 (s, 12H, CH$_3$), 2.55 (s, 24H, CH$_3$), 3.09

15   (m, 12H, NCH$_2$), 3.72 (d, J=4.53 Hz, 4H, NCH$_2$), 5.48 (t, J=4.31 Hz, 2H, CH=CH), 6.92 (s, 4H, Ph), 6.93 (s, 4H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.71, 20.90, 22.71, 22.76, 24.74, 40.04, 42.21, 44.56, 45.69, 128.45, 131.88, 132.02, 140.05, 140.16, 142.20, 142.58. MS-FAB (m/z) 1012.8 (M$^+$, 100%), 828.7, 646.7, 561, 176.

Compound 7 was obtained from **6** as described elsewhere (Reddy et al., *J. Med. Chem.*

20   41:4723 (1998)) in 75% yield, mp >230°C. ^{1}H-NMR (D$_2$O): δ 1.26 (t, J=12.5 Hz, 6H, 2CH$_3$), 1.79 (m, 8H, CH$_2$), 3.12 (m, 12H, NCH$_2$), 3.80 (d, J=7.16, 4H, NCH$_2$), 6.10 (m, 2H, CH=CH); ^{13}C-NMR (D$_2$O): δ 12.79, 25.10, 45.19, 48.53, 48.62, 50.36, 130.66. MS-MALDI (m/z): 285.3 (MH$^+$, 100%).

Compound 8 was obtained from the commercially available butyne diol.

25   Mesitylenesulfonyl chloride (19.5 g, 90 mmol) in dioxane (30 ml) was added dropwise to a stirred and cooled mixture of butyne diol (2.58 g, 30 mmol), 50% potassium hydroxide (30 ml) and triethylbenzyne ammonium bromide (405 mg, 1.5 mmol). Once the addition was over, the mixture was stirred at room temperature for an additional 3 h. An excess of water was added and the white precipitate was cooled over night, filtered off and dried.

30   Recrystallization from ethyl acetate/hexane afforded 8.6 g (63%) of **8**; mp 105-106°C.

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^{1}H-NMR (CDCl$_3$): δ 2.30 (s, 6H), 2.60 (s, 12H), 4.50 (s, 4H), 6.95 (s, 4H); ^{13}C-NMR (CDCl3): δ 20.93, 22.48, 56.13, 80.41, 130.65, 131.67, 139.98, 143.67. MS-EI (m/z) 450 (M$^+$).

Compound 9 was obtained following a procedure analogous to that described for compound **42** (see below). From 450 mg (1 mmol) of diester **8** and 1.05 g (2.2 mmol) of diamide **4**, 570 mg (56%) of tetramide **9** was obtained. ^{1}H-NMR (CDCl$_3$): δ 0.90 (t, 6H), 1.30 (bs, 8H), 2.20 (s, 12H), 2.45 (s, 24H), 3.05 (m, 12H), 3.75 (s, 4H), 6.87 (s, 8H); ^{13}C-NMR (CDCl$_3$): δ 12.70, 20.78, 22.68, 34.65, 39.97, 44.46, 44.99, 78.62, 131.85, 131.98, 132.34, 140.14, 142.13, 142.55. MS-FAB (m/z) 1010 (M$^\oplus$).

Compound 10 was obtained following a procedure analogous to that described for compound **43** (see below). From 500 mg (0.49 mmol) of tetramide **9**, 160 mg (76%) of the tetrahydrochloride **25** was obtained; mp >280°C (decomp). ^{1}H-NMR (D$_2$O): δ 1.30 (t, 6H), 1.80 (b, 8H), 2.90-3.25 (m, 12H), 4.05 (s, 4H); ^{13}C-NMR (D$_2$O): δ 13.39, 25.64, 39.26, 45.72, 49.00, 49.20, 81.20. MS-MALDI 283 (M$^+$ + 1).

Compound 11: Mesitylenesulfonylethylamide **1** (3.1 g, 13.65 mmol) was dissolved in anhydrous DMF (30 ml) followed by the addition of NaH (85%, 0.423 g) in several portions. The mixture was stirred at room temperature for 1 h. N-(4-chloro-2-butenyl)-phthalimide (Aldrich, 3.06 g, 13 mmol) in 20 ml of DMF was added to the flask followed by stirring at 80°C over night. The mixture was cooled to room temperature, quenched with H$_2$O (10 ml), and the solution was evaporated to dryness in vacuo. The solid residue was partitioned between 25 ml H$_2$O and 25 CHCl$_3$. The aqueous layer was extracted with CHCl$_3$ (3 x 25 ml), the organic layers were washed with brine (35 ml), dried (MgSO$_4$), the solvent was evaporated to afford a gum which solidified upon trituration with hexane to give **11**. The ^{1}H-NMR and ^{13}C-NMR spectra showed that **11** was pure enough to be used in the next step without further purification, yield 4.75 g. ^{1}H-NMR (CDCl$_3$): δ 1.16 (t, J=7.11 Hz, 3H, CH$_3$), 2.29 (s, 3H, CH$_3$), 2.63 (s, 6H, 2CH$_3$), 3.29 (q, J=7.11 Hz, 2H, CH$_2$), 4.06 (d, J=5.24 Hz, 2H, NCH$_2$), 4.26 (d, J=5.72 Hz, 2H, NCH$_2$), 5.59 (m, 2H, CH=CH), 6.95 (s, 2H, Ph), 7.71 (m, 2H, Ph), 7.83 (m, 2H, Ph); ^{13}C-NMR (CDCl$_3$): δ 13.06, 20.89, 22.72, 34.35, 40.68, 42.01, 123.27, 126.69, 129.47, 131.90, 134.00, 140.24.

Compound 12: Amide **11** (20 g, 46.95 mmol) was dissolved in methanol, hydrazine monohydrate (5 ml, 98.52 mmol) was added and the solution stirred at 55°C for 24 h. Initially it was a homogeneous solution; however, after several hours a white solid precipitated. The mixture was cooled to room temperature, 300 ml of conc. HCl were added slowly (exothermic reaction), and stirring at room temperature was continued for 12 h more. Methanol was evaporated, and the resulting solid was extracted with $CHCl_3$ (3 x 150 ml). The aqueous layer was neutralized with 50% NaOH, extracted again with $CHCl_3$ (3 x 100 ml), the combined organic layers were dried ($MgSO_4$); the solution was evaporated to afford a gum, which solidified under high vacuum to give **12**; yield 9.0 g (65%). The compound was purified by column chromatography using hexane, ethyl acetate (7:3) as eluent; mp 167-169°C. ^{1}H-NMR ($CDCl_3$): δ 1.0 (t, J=7.1 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.56 (s, 6H, $2CH_3$), 2.62 (br, NH_2), 3.12 (q, J=7.1 Hz, 2H, NCH_2), 3.73 (br, 2H, NCH_2), 3.94 (d, J=6.0 Hz, 2H, NCH_2), 5.80 (m, 2H, CH=CH), 6.92 (s, 2H, Ph); ^{13}C-NMR ($CDCl_3$): δ 12.97, 20.93, 22.74, 36.43, 40.94, 42.08, 124.29, 131.89, 132.00, 132.62, 140.21, 142.67.

Compound 13 was obtained from **12** as described for **4** in 96% yield. It was purified by column chromatography using hexane and ethyl acetate (4:1.5) as eluants; mp 98-99°C; ^{1}H-NMR ($CDCl_3$): δ 0.93 (t, J=5.85 Hz, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 2.56 (s, 6H, $2CH_3$), 3.06 (q, J=7.15 Hz, 2H, NCH_2), 3.48 (t, J=5.99 Hz, 2H, NCH_2), 3.68 (d, J=5.72 Hz, 2H, NCH_2), 4.58 (t, J=6.24 Hz, 1H, NH), 5.44 (m, 2H, CH=CH), 6.87 (s, 2H, Ph), 6.89 (s, 2H, Ph); ^{13}C-NMR ($CDCl_3$): δ 12.80, 20.89, 22.64, 22.89, 39.01, 40.59, 41.41, 128.14, 128.46, 131.91, 131.96, 139.08, 140.19, 142.26, 142.54. MS-FAB (m/z) 479.2 (M^+, 65%), 296.2, 279.1, 267.2, 183.1.

Compound 15: Amide **13** (4.79 g, 10 mmol) was dissolved in anhydrous DMF (40 ml) followed by addition of NaH (0.37 g) in several portions, the mixture stirred at room temperature for 2 h, cis-1,4-dichloro-2-butene (7.5 g, 60 mmol) in 10 ml DMF was added at once, and stirring was continued at 50°C over night. The mixture was cooled to room temperature, quenched with 10 ml H_2O, the solvents were evaporated, and the contents were partitioned between H_2O (50 ml) and $CHCl_3$ (50 ml). The aqueous layer was extracted with $CHCl_3$ (3 x 50 ml), the pooled organic layers were dried ($MgSO_4$),

40

evaporated, and **15** was purified by column chromatography using hexane, ethyl acetate (8.5:1.5) as eluants; yield 5.5 g (97%), mp 106-108°C. ^{1}H-NMR (CDCl$_3$): δ 1.03 (t, J=7.33 Hz, 3H, CH$_3$), 2.30 (s, 6H, 2CH$_3$), 2.57 (s, 12H, 4CH$_3$), 3.17 (q, J=7.31 Hz, NCH$_2$), 3.71 (m, 4H, NCH$_2$), 3.81 (d, J=6.87 Hz, 2H, NCH$_2$), 3.95 (d, J=7.70 Hz, 2H,

5   CHCl$_2$), 5.50 (m, 3H, CH=CH), 5.74 (m, 1H, CH=CH), 6.93 (s, 2H, Ph), 6.95 (s, 2H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.91, 22.70, 22.74, 38.20, 40.45, 41.60, 42.11, 42.33, 128.17, 128.95, 129.34, 129.40, 131.94, 132.08, 140.23, 140.34, 142.91. MS-FAB (m/z) 566.7 (M$^+$, 100%), 153.4, 96.3.

Compound 14 was prepared from **13** and 1,4-diiodobutane as described above for **15**.

10   The product was purified by column chromatography using hexanes and ethyl acetate (4:1) as eluant; yield 79%. ^{1}H-NMR (CDCl$_3$): δ 1.04 (t, J=7.10 Hz, 3H, CH$_3$), 1.63 (m, 4H, CH$_2$), 2.30 (s, 6H, 2CH$_3$), 2.58 (s, 12H, 4CH$_3$), 3.04 (t, J=6.50 Hz, 2H, CH$_2$I), 3.16 (m, 4H, NCH$_2$), 3.78 (d, J=5.14 Hz, 4H, NCH$_2$), 5.55 (m, 2H, CH=CH), 6.94 (s, 2H, Ph), 6.95 (s, 2H, Ph); ^{13}C-NMR (CDCl$_3$): δ 5.69, 12.92, 20.95, 22.72, 22.78, 28.25, 30.36,

15   40.47, 41.59, 42.11, 44.71, 128.34, 129.00, 131.94, 132.06, 132.60, 132.89, 140.15, 140.21, 142.50, 142.71.

Compound 16 was prepared from **4** and 4-bromobutyronitrile as described above for Compound **2** in 94% yield.

^{1}H NMR(CDCl$_3$): δ 0.97 (t, J = 7.12Hz, 3H, CH$_3$), 1.40 (m, 4H, 2CH$_2$), 1.85 (Pent., m,

20   2H, CH$_2$), 2.27 (t, J = 7.17Hz, 2H CH$_2$CN), 2.30 (s, 6H, 2CH$_3$), 2.57 (s, 6H, 2CH$_3$), 2.58 (s, 6H, 2CH$_3$), 3.13 (m, 6H, NCH$_2$), 3.28 (t, J = 7.11Hz, 2H, NCH$_2$), 6.94 (s, 2H, Ph), 6.96 (s, 2H, Ph); ^{13}C NMR (CDCl$_3$): δ 12.55, 14.54, 20.84, 22.64, 22.73, 23.65, 24.43, 24.57, 39.88, 44.31, 44.54, 45.58, 118.69, 131.84, 132.05, 132.73, 133.36, 139.94, 142.20, 142.71.

25   **Compound 17** was prepared from **16** as described above for Compound **3** in 93% yield.
^{1}H NMR(CDCl$_3$): δ 1.00 (t, J = 6.92Hz, 3H, CH$_3$), 1.40 (m, 10H, 4CH$_2$, NH$_2$), 2.29 (s, 6H, 2CH$_3$), 2.57 (b, 14H, 4CH$_3$, CH$_2$N), 3.13 (m, 8H, 4CH$_2$N), 6.93 (s, 4H, 2 Ph); ^{13}C NMR (CDCl$_3$): 12.72, 20.90, 22.72, 22.78, 24.67, 24.80, 30.80, 40.02, 41.61, 44.56, 45.10, 45.38, 131.87, 140.04, 142.21, 142.28; MS-FAB(M/Z) 552.3(M$^+$, 100%), 368.2,

30   299.1, 183.0, 154.0.

Compound **18** was prepared from **17** as described above for Compound **4**.

^{1}H NMR(CDCl$_3$): δ 0.96 (t, J = 7.13Hz, 3H, CH$_3$), 1.38 (m, 8H, 4CH$_2$), 2.29 (s, 9H, 3CH$_3$), 2.55 (s, 6H, 2CH$_3$), 2.56 (s, 6H, 2CH$_3$); 2.59 (s, 6H, 2CH$_3$), 2.80 (m, 2H, CH$_2$N), 3.10 (m, 8H, NCH$_2$), 4.67(t, J = 6.6Hz, 1H, NH), 6.93 (s, 6H, 3 Ph); ^{13}C NMR(CDCl$_3$): δ

5   12.56, 20.87, 22.70, 22.74, 22.84, 24.40, 26.45, 24.67, 26.62, 39.87, 41.88, 44.45, 45.02, 45.09, 131.86, 131.90, 131.92, 133.12, 133.32, 133.68, 138.91, 139.97, 142.02, 142.21, 142.38; MS-FAB(M/Z): 756.9(M + 23(Na), 100%) 572.8, 390.7, 333.6, 305.6

Compound **19** was prepared from **4** and 1,4-dichloro-2-butene as described above for **15** in 99% yield. ^{1}H-NMR (CDCl$_3$): δ 1.01 (t, J=7.11 Hz, 3H, CH$_3$), 1.38 (m, 4H, CH$_2$),

10   2.29 (s,3H), 2.30 (s,3H), 2.57 (s, 6H), 2.61 (s, 6H), 3.11 (m, 4H, NCH$_2$), 3.16 (q, J=7.15 Hz, 2H, NCH$_2$), 3.81 (d, J=7.17 Hz, 2H, NCH$_2$), 3.98 (d, J=8.05 Hz, 2H, CH$_2$Cl), 5.51 (m, 1H, CH=CH), 5.77 (m, 1H, CH-CH), 6.93 (s, 2H, Ph), 6.95 (s, 2H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.76, 20.91, 22.71, 22.76, 24.74, 38.12, 40.08, 41.85, 44.59, 45.54, 129.14, 129.25, 131.88, 132.02, 140.09, 140.19, 142.21, 142.63. MS-FAB (m/z) 569.3 (M$^+$,

15   20%), 385.2, 240.1, 203.3, 183.0, 119 (100%).

Compound **20** was prepared from **18** and **15** following the procedure described above for **15**. It was purified by column chromatography using hexanes - ethyl acetate (7:3) as eluant (78% yield). ^{1}H-NMR (CDCl$_3$): δ 0.97 (t, J=7.10 Hz, 3H, CH$_3$), 0.99 (t, J=7.0 Hz, 3H, CH$_3$), 1.29 (m, 8H, CH$_2$), 2.29 (s, 15H, CH$_3$), 2.54, 2.55, 2.59 (s, 30H, CH$_3$), 3.06

20   (m, 12H, NCH$_2$), 3.65 (m, 8H, NCH$_2$), 5.48 (m, 4H, CH=CH), 6.92 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.70, 12.83, 20.88, 20.91, 22.65, 22.68, 22.72, 22.74, 24.48, 24.72, 40.04, 40.47, 41.53, 42.07, 42.22, 42.34, 44.54, 44.96, 127.94, 128.27, 128.57, 129.20, 131.92, 132.05, 139.96, 140.00, 140.12, 140.16, 140.27, 142.19, 142.25, 142.47, 142.58, 142.87. MS-FAB (m/z) 1263.81 (M$^+$, 100%), 1080.01, 898.11, 714.81, 563.

25   Compound **21**: Pentamide **20** (0.93 g, 0.735 mmol) was dissolved in 20 ml anhydrous CH$_2$Cl$_2$, phenol (3.46 g, 36.77 mmol) was added, followed by HBr in acetic acid (30%, 17.62 ml) and the mixture was stirred over night at 25°C. Water (10 ml) was added to the flask, the aqueous layer was separated, the organic layer was extracted with 5 ml H$_2$O, and the combined aqueous layers were washed with CH$_2$Cl$_2$ (6 x 15 ml). Water was

30   evaporated under vacuum to afford a solid which was dissolved in 1 ml 1N NaOH

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followed by 1 ml of 50% KOH. This solution was extracted with CHCl₃ (10 x 5 ml).
The combined organic layers were dried (MgSO₄), CHCl₃ was evaporated, and the
residue dissolved in anhydrous diethyl ether. Anhydrous HCl gas was passed into the
solution while cooling at 0°C. A white solid precipitated which was filtered and washed

5   with ether. It was **21** (84%). ^{1}H-NMR (D₂O): δ 1.29 (t, J=7.32 Hz, 3H, CH₃), 1.31 (t,
J=7.24 Hz, 3H, CH₃), 1.79 (m, 8H, CH₂), 3.12 (m, 12H, NCH₂), 3.87 (m, 8H, NCH₂),
5.98 (m, 4H, CH=CH); ^{13}C-NMR (D₂O): δ 13.36, 13.46, 25.66, 25.77, 45.44, 45.74,
46.24, 46.41, 46.84, 49.09, 49.41, 49.70, 129.02, 129.16, 129.47, 129.66. MS-MALDI
(m/z) 354.36 (MH$^+$, 100%).

10 **Compound 22** was prepared in 51% yield from **18** and **14** as described above for
compound **15**. ^{1}H-NMR (CDCl₃): δ 0.97 (t, J=6.59 H, 3H, CH₃), 0.99 (t, J=7.02 Hz, 3H,
CH₃), 1.29 (m, 12H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s), 2.56 (s), 2.57 (s), 3.10 (m, 16H,
NCH₂), 3.70 (m, 4H, NCH₂), 5.47 (m, 2H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C-NMR
(CDCl₃): δ 12.69, 12.83, 20.91, 22.69, 22.71, 22.76, 24.43, 24.70, 40.48, 41.11, 41.48,
15 44.50, 44.91, 128.13, 128.90, 131.88, 131.94, 132.01, 133.29, 139.95, 140.00, 140.15,
142.22, 142.29, 142.60. MS-FAB (m/z) 1265.91 (M$^+$, 100%), 1082.01, 900.11, 716.91,
563.81.

Compound 23 was prepared from **22** in 79% yield as described above for **21**. ^{1}H-NMR
(D₂O): δ 1.29 (t, J=7.29 Hz, 3H, CH₃), 1.30 (t, J=7.30 Hz, 3H, CH₃), 1.78 (m, 12H, CH₂),
20 3.12 (m, 16H, NCH₂), 3.83 (m, 4H, NCH₂), 5.96 (m, 2H, CH=CH); ^{13}C-NMR (D₂O): δ
13.31, 13.42, 25.62, 25.75, 45.38, 45.71, 46.18, 46.76, 49.07, 49.32, 49.69, 129.11,
129.39. MS-MALDI (m/z) 356.38 (MH$^+$, 100%).

Compound 24 was prepared from **18** (52% yield) as described. ^{1}H-NMR (CDCl₃): δ
0.95 (m, 6H, 2CH₃), 1.32 (m, 12H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s, 30H, CH₃), 3.06
25 (m, 16H, NCH₂), 3.70 (m, 4H, NCH₂), 5.47 (m, 2H, CH=CH), 6.92 (s, 10H, Ph); ^{13}C-
NMR (CDCl₃): δ 12.67, 20.90, 22.71, 22.76, 24.43, 24.68, 39.97, 42.08, 44.48, 44.90,
45.61, 128.28, 128.45, 131.87, 131.93, 132.01, 139.96, 140.00, 140.12, 142.21, 142.28,
142.58. MS-FAB (m/z) 1265.91 (M$^+$, 100%), 1082.01, 900.11.

Compound 25 was prepared from **24** in 96% yield as described above for **21**. ^{1}H-NMR
30 (D₂O): δ 1.28 (t, J-7.29 Hz, 6H, 2CH₃), 1.78 (m, 12H, CH₂), 3.09 (m, 16H, NCH₂), 3.84

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(m, 4H, NCH$_2$), 5.96 (m, 2H, CH=CH); ^{13}C-NMR (D$_2$O): δ 13.31, 25.61, 25.73, 45.70, 46.79, 49.05, 49.36, 49.65, 129.19. MS-MALDI (m/z) 356.4 (MH$^+$).

Compound 26: A mixture of KOH (0.25 g), K$_2$CO$_3$ (0.25 g) and tetra-n-butyl-ammonium hydrogen bromide (0.05 g) were suspended in 15 ml benzene.

5 Mesitylenesulfonylamide (0.199 g, 1 mmol) was added to the suspension and the mixture was heated to 50°C. Iodide **14** (1.98 g, 3 mmol) in 10 ml benzene was added to the flask, the mixture heated under reflux over night, then cooled to room temperature; the inorganic solids were filtered off and washed with benzene (2 x 20 ml). The combined organic layers were washed several times with water until the washings were neutral.

10 The benzene was dried (MgSO$_4$), evaporated and the residue purified by column chromatography using hexanes and ethyl acetate (7.5:2.5) as eluant; 25% yield (0.948 g). ^{1}H-NMR (CDCl$_3$): δ 1.00 (t, J=7.18 Hz, 6H, CH$_3$), 1.28 (m, 8H, CH$_2$), 2.29 (s, 15H, CH$_3$), 2.53 (s), 2.55 (s), 2.57 (s), 3.03 (m, 8H, NCH$_2$), 3.12 (q, J=7.13 Hz, 4H, NCH$_2$), 3.70 (m, 8H, NCH$_2$), 5.47 (m, 4H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ

15 12.78, 20.85, 22.63, 22.69, 24.32, 24.58, 40.41, 41.43, 42.00, 44.76, 45.43, 128.08, 128.83, 131.88, 131.95, 132.77, 132.85, 133.23, 139.90, 140.04, 140.08, 142.22, 142.43, 142.53. MS-FAB (m/z) 1263.81 (M$^+$, 100%), 1081, 898.11, 815.01, 561.81, 418.81.

Compound 27 was prepared from **26** in 57% yield as described above for **21**. ^{1}H-NMR (D$_2$O): δ 1.31 (t, J=7.31 Hz, 6H, CH$_3$), 1.78 (m, 8H, CH$_2$), 3.15 (m, 12H, NCH$_2$), 3.83

20 (m, 8H, NCH$_2$), 5.96 (m, 4H, CH=CH); ^{13}C-NMR (CDCl$_3$): δ 13.43, 25.64, 25.76, 45.39, 46.19, 46.77, 49.35, 49.72, 129.11, 129.41. MS-MALDI (m/z) 354.3 (MH$^+$, 100%).

Compound 28 was prepared from **15** and mesitylenesulfonylamide in 24% yield as described above for **26**; mp 57.7°C. ^{1}H-NMR (CDCl$_3$): δ 0.99 (t, J=7.09 Hz, 6H, CH$_3$), 2.29 (s, 15H, CH$_3$), 2.53 (s), 2.55 (s), 3.12 (q, J=7.09 Hz, 4H, NCH$_2$), 3.63 (m, 16H,

25 NCH$_2$), 5.49 (m, 8H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.85, 20.89, 20.92, 22.66, 40.47, 41.53, 42.19, 128.00, 128.47, 128.58, 129.11, 131.92, 132.05, 140.17, 140.30, 142.46, 142.87. MS-FAB (m/z) 1259.81 (M$^+$, 60%), 1075.91, 894.01, 306.51, 153.4 (100%).

Compound 29 was prepared from **28** in 81% yield as described above for **21**. ^{1}H-NMR (D$_2$O): δ 1.31 (t, J=7.29 Hz, 6H, CH$_3$), 3.15 (q, J=7.31 Hz, 4H, NCH$_2$), 3.84 (m, 4H,

NCH$_2$), 3.90 (m, 12H, NCH$_2$), 5.98 (m, 8H, CH=CH); ^{13}C-NMR (D$_2$O): δ 13.42, 45.41, 46.22, 46.44, 129.07, 129.37, 129.42, 129.58. MS-MALDI (m/z) 350.31 (MH$^+$).

Compound 30 was prepared from **19** in 25% yield as described above for **26**; mp 62.3°C. ^{1}H-NMR (CDCl$_3$): δ 0.95 (5, J=7.17 Hz, 6H, CH$_3$), 1.33 (m, 8H, CH$_2$), 2.29 (s, 15H, CH$_3$), 2.54 (s), 2.55 (s), 3.07 (m, 12H, NCH$_2$), 3.65 (m, 8H, NCH$_2$), 5.48 (m, 4H, CH=CH), 6.92 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.69, 20.90, 22.69, 22.73, 24.70, 40.03, 42.13, 42.30, 44.53, 45.59, 128.11, 128.79, 131.87, 132.00, 140.02, 140.14, 140.28, 142.17, 142.58, 142.85. MS-FAB (m/z) 1263.81 (M$^+$, 100%), 1080.01, 898.11, 714.01, 153.

Compound 31 was prepared from **30** in 87% yield as described above for **21**. ^{1}H-NMR (D$_2$O): δ 1.28 (t, J=7.32 Hz, 6H, CH$_3$), 1.79 (m, 8H, CH$_2$), 3.10 (m, 12H, NCH$_2$), 3.87 (m, 8H, NCH$_2$), 5.98 (m, 4H, CH=CH), ^{13}C-NMR (D$_2$O): δ 12.70, 25.00, 25.13, 45.10, 45.81, 46.21, 48.44, 48.78, 128.44, 128.85. MS-MALDI (m/z) 354.3 (MH$^+$).

Compound 32: Mesitylenesulfonylamide (1.47 g, 7.38 mmol) was dissolved in 50 ml anhydrous DMF, and NaH (85%, 0.3 g) was added to it under a nitrogen atmosphere. The mixture was stirred at room temperature and **19** (1.40 g, 2.46 mmol) in 25 ml DMF were added. Heating at 65°C continued over night. The mixture was cooled to room temperature, and 10 ml of H$_2$O were added. The solvents were evaporated and the solid residue was partitioned between 40 ml H$_2$O and 40 ml CHCl$_3$. The aqueous layer was extracted with CHCl$_3$ (2 x 30 ml), the combined organic layers were washed with H$_2$O (3 x 50 ml), dried (MgSO$_4$), and evaporated. The residue was purified by column chromatography using hexanes - ethyl acetate (7.5:2.5). 1.7 g (97%) of **32** as a white solid was obtained. ^{1}H-NMR (CDCl$_3$): δ 0.94 (t, J=7.10 Hz, 3H, CH$_3$), 1.30 (m, 4H, CH$_2$), 2.29 (s), 2.30 (s), 2.55 (s, 12H, CH$_3$), 2.65 (s, 6H, CH$_3$), 3.11 (m, 6H, NCH$_2$), 3.52 (m, 1H, NCH), 3.65 (m, 2H, NCH$_2$), 3.71 (m, 1H, NCH$_2$), 4.82 (br, 1H, NH), 5.47 (m, 2H, CH=CH), 6.93 (s, 4H, Ph), 6.96 (s, 2H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.50, 20.91, 22.71, 22.76, 22.83, 22.91, 24.66, 38.98, 39.85, 42.15, 42.26, 44.50, 128.06, 128.51, 131.86, 131.91, 138.18, 140.00, 140.14, 140.28, 142.17, 142.65.

Compound 33 was prepared from **32** and **14** in 51% yield as described above for **22**. ^{1}H-NMR (CDCl$_3$): δ 0.99 (5, J=7.19 Hz, 6H, CH$_3$), 1.33 (m, 8H, CH$_2$), 2.29 (s, 15H, CH$_3$),

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2.55 (s), 2.57 (s), 3.10 (m, 12H, NCH$_2$), 3.70 (m, 4H, NCH$_2$), 3.77 (m, 4H, NCH$_2$), 5.42 (m, 4H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.70, 12.71, 20.89, 22.66, 22.72, 22.78, 22.81, 24.60, 26.53, 40.39, 41.37, 41.87, 42.20, 45.47, 128.26, 128.62, 131.78, 131.84, 131.86, 131.92, 132.77, 138.92, 139.96, 140.09, 140.17, 142.57, 142.63.

5   **Compound 34** was prepared from **33** as described above for **21** in 40% yield.

Compound 35 was prepared from **15** in 94% yield as described above for **32**.

Compound 36 was prepared from **35** and **14** in 82% yield as described above for **33**. ^{1}H-NMR (CDCl$_3$): δ 0.99 (t, J=7.11 Hz, 6H, CH$_3$), 1.33 (m, 4H, CH$_2$), 2.29 (s, 15H, CH$_3$), 2.55 (s), 2.57 (s), 3.07 (m, 8H, NCH$_2$), 3.70 (m, 12H, NCH$_2$), 5.46 (m, 6H, CH=CH),

10   6.92 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.69, 12.80, 20.84, 22.62, 22.68, 22.73, 22.77, 24.58, 26.55, 40.44, 41.51, 41.86, 42.04, 42.24, 45.49, 128.10, 128.25, 128.52, 128.62, 128.82, 131.89, 131.95, 132.79, 138.89, 140.07, 140.14, 140.23, 141.94, 142.44, 142.53, 142.82. MS-FAB (m/z) 1262.8 (M$^+$, 75%), 1080.01, 896, 119 (100%).

Compound 37 was prepared from **36** in 65% yield as described above for **21**. ^{1}H-NMR

15   (D$_2$O): δ 1.31 (t, J=6.97 Hz, 6H, CH$_3$), 1.79 (m, 4H, CH$_2$), 3.12 (m, 8H, NCH$_2$), 3.83 (m, 12H, NCH$_2$), 5.96 (m, 6H, CH=CH); ^{13}C-NMR (D$_2$O): δ 13.48, 25.69, 26.76, 41.67, 45.44, 46.24, 46.45, 46.80, 49.41, 129.00, 129.12, 129.45, 129.71. MS-MALDI (m/z) 352.3 (MH$^+$).

Compound 38 was prepared from **35** and **19** in 89% yield as described. ^{1}H-NMR

20   (CDCl$_3$): δ 0.95 (m, 6H, CH$_3$), 1.33 (m, 4H, CH$_2$), 2.29 (s, 15H, CH$_3$), 2.54 (s), 2.55 (s), 2.57 (s), 3.09 (m, 8H, NCH$_2$), 3.66 (m, 12H, NCH$_2$), 5.48 (m, 6H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C-NMR (CDCl$_3$): δ 12.51, 12.63, 20.84, 20.86, 22.63, 22.65, 22.84, 24.61, 38.92, 40.40, 41.40, 42.11, 42.18, 44.44, 45.48, 127.95, 128.07, 128.49, 128.62, 128.80, 131.76, 131.83, 131.85, 131.88, 132.01, 138.05, 139.01, 140.07, 140.13, 140.24, 142.15, 142.21,

25   142.87. MS-FAB (m/z) 1263.1 (M$^+$, 90%), 1080.1, 896.01, 119 (100%).

Compound 39 was prepared from **38** in 54% yield as described above for **21**; mp 270°C (dec.). ^{1}H-NMR (D$_2$O): δ 1.31 (m, 6H, CH$_3$), 1.80 (m, 4H, CH$_2$), 3.10 (m, 8H, NCH$_2$), 3.86 (m, 12H, NCH$_2$), 5.98 (m, 6H, CH=CH); ^{13}C-NMR (D$_2$O): δ 13.30, 13.42, 25.58, 25.70, 45.69, 46.21, 46.43, 46.81, 49.02, 49.37, 129.00, 129.15, 129.37, 129.59. MS-

30   MALDI (m/z): 352.343 (MH$^+$).

Compound 42: NaH (80%, 132 mg, 4.4 mmol) was added to a solution of diamide **41**
(1.98 g, 4.4 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 minutes and
a solution of the diester **40** (Reddy et al. (1998) J. Med Chem., 41:4723) (960 mg, 2
mmol) in DMF (10 ml) was added dropwise. The mixture was stirred at 75°C for 2 h, the

5   solvent was distilled off, the residue was taken in chloroform, washed with a saturated
solution of ammonium chloride, dried (Na_2SO_4) and evaporated to dryness. The crude oil
was purified by column chromatography using hexane - ethyl acetate (8:2) as running
solvent. 1.4 g (70%) was obtained as a glassy oil. ^{13}C-NMR ($CDCl_3$): δ 20.58, 22.63,
22.80, 32.42, 33.86, 43.16, 45.42, 46.26, 132.75, 133.21, 139.82, 142.40. MS-FAB 984

10   (M^+),

Compound 43: Phenol (1.86 g, 19.7 mmol) and 30% HBr in glacial acetic acid (35 ml)
were added in that order to a solution of **42** (600 mg, 0.6 mmol) in CH_2Cl_2 (35 ml) at
room temperature. The solution was stirred for 24 h, water (30 ml) was added, followed
by extraction with methylene chloride (3 x 20 ml). The aqueous layer was evaporated

15   under reduced pressure and the residue was taken up in 2N NaOH (2 ml) and then 50%
KOH (2 ml) followed by extraction with chloroform (6 x 10 ml). After removal of
chloroform, the residue was taken up in ethanol (15 ml) and acidified with concentrated
hydrochloric acid (0.4 ml). The product **43** (230 mg, 93%) was recrystallized from
aqueous ethanol; mp >270°C (decomp). ^{1}H-NMR (D_2O): δ 1.95 (m, 2H), 2.05-2.25 (m,

20   6H), 2.75 (s, 6H), 2.90 (b, 2H), 3.10-3.35 (m, 12H); ^{13}C-NMR (D_2O): δ 25.21, 25.24,
35.60, 35.64, 47.41, 48.58, 50.87. MS-MALDI (m/z) 240 (M^+ + 1).

Compound 47: NaH (80%, 132 mg, 4.4 mmol) was added to a solution of diamide **46**
(1.98 g, 4.4 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 min and a
solution of the diester **8** (900 mg, 2 mmol) in DMF (10 ml) was added dropwise. The

25   mixture was stirred at 75°C for 2 h. The solvent was distilled off, the residue was taken
up in chloroform, washed with a saturated solution of ammonium chloride, dried ($NaSO_4$)
and evaporated to dryness. The oily residue was crystallized from ethyl acetate/hexane
1.2 g (61%); mp 165-166°C. ^{1}H-NMR ($CDCl_3$): δ 1.08 (t, 3H), 1.75 (m 4H), 2.28 (s,
12H), 2.55 (bs, 24H), 3.10 (m, 12H), 3.98 (s, 4H), 6.95 (m, 8H); ^{13}C-NMR ($CDCl_3$): δ

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12.70, 20.86, 22.64, 25.14, 34.85, 40.22, 42.62, 43.37, 78.80, 131.99, 132.26, 133.21, 140.26, 142.28, 142.71. MS-FAB (m/z) 982 (M^+).

Compound 48 was obtained as described for **47**. From 1.2 g (1.22 mmol) of tetramide **47**, 420 mg (86%) of the tetrahydrochloride **48** was obtained; mp >270°C (decomp). ^{1}H-NMR (D_2O): δ 1.29 (t, 6H), 2.13 (m, 4H), 3.14 (m, 12H), 4.06 (s, 4H); ^{13}C-NMR (D_2O): δ 13.34, 25.52, 39.45, 45.90, 45.64, 46.71, 81.32. MS-MALDI (m/z) 255 (M^+ + 1).

Compound 44 was obtained as described for **47**. From 450 mg (1 mmol) of diester **8** and 994 mg (2.2 mmol) of diamide **41**, 500 mg (52%) of the tetramide **44** was obtained and crystallized from ethyl acetate - hexane; mp 155-156°C.

Compound 45 was obtained as described for **43**. From 500 mg (0.52 mmol) of tetramide **44**, 160 mg (82%) of the tetrahydrochloride **45** was obtained; mp >270°C (decomp). ^{1}H-NMR (D_2O): δ 2.15 (m, 4H), 2.73 (s, 3H), 3.05-3.40 (m, 8H), 4.10 (s, 4H); ^{13}C-NMR (D_2O): δ 25.59, 35.66, 45.90, 46.57, 48.61.

Compound 51 is a mixture of *cis/trans-* isomers. ^{1}H-NMR (D_2O): δ 1.15-2.10 (m, 7H), 2.90 (q, 1H), 3.30-3.80 (b, 2H); ^{13}C-NMR (D_2O): δ 24.16, 24.97, 28.44, 30.42, 36.58, 37.14, 48.24, 52.27, 55.19, 57.45, 64.55, 67.26.

Compound 52: Mesitylenesulfonylchloride (6.5 g, 30 mmol) in dioxane (10 ml) was added dropwise to a stirred and cooled mixture of amine alcohol **51** (1.15 g, 10 mmol), triethylbenzyl ammonium bromide (135 mg, 0.5 mmol), 50% KOH (10 ml) and dioxane (10 ml). The reaction mixture was left over night at 20 °C with magnetic stirring. An excess of water was added, the solution was extracted with chloroform (3 x 30 ml), dried (Na$_2$SO$_4$) and evaporated to dryness. The oily residue was chromatographed on a silica-gel column using hexane:ethyl acetate (8:2) as eluants. Crystallization from ethyl acetate - hexane afforded 1.2 g (25%) of pure **52**; mp 167-168°C. ^{1}H-NMR (CDCl$_3$): δ 1.35-1.90 (6H), 1.90-2.15 (m, 1H), 2.30, 2.35 (s, 6H), 2.65 (s, 12H), 3.20 (m, 1H), 3.70 (m, 1H), 3.90 (m, 1H), 5.15 (d, 1H), 6.90, 7.00 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 20.73, 20.85, 22.15, 22.37, 22.70, 26.94, 32.75, 45.34, 56.09, 70.38, 130.22, 131.57, 133.98, 138.68, 139.64, 142.02, 143.10. MS-EI (m/z) 479 (M^+), 280 ($M^{\oplus}$ - 199).

Compound 54: NaH (105 mg, 3.5 mmol) was added to a solution of compound **52** (1.7 g, 3.5 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 min and a solution

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of compound **53** (1.34 g, 3.85 mmol) in DMF (5 ml) was added in small portions. The mixture was stirred at 75 °C for 2 h. The solvent was distilled off, the residue was taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na$_2$SO$_4$) and evaporated. The oily residue was purified by column chromatography

5 (hexane - ethyl acetate 8:2) which gave compound **54** (1.22 g, 47%). ^{1}H-NMR (CDCl$_3$): δ 1.98 (t, 3H), 1.20-2.05 (9H), 2.20 (s, 6H), 2.55, 2.65 (s, 12H), 2.70-3.55 (9H), 6.85 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 12.49, 20.80, 21.64, 21.87, 22.88, 28.72, 33.16, 36.13, 39.96, 43.80, 47.95, 57.77, 61.26, 131.83, 132.94, 133.14, 138.82, 139.90, 142.07, 142.63. MS-FAB (m/z) 628 (M$^+$ + 1), 546 (M$^+$ - 81).

10 **Compound 55** was obtained following the procedure described for compound **42**. From 1.22 g (1.6 mmol) of bromoderivative **54** and 820 mg (1.76 mmol) of diamide **46**, 1.26 g (77%) of tetramide **55** was obtained as a glassy oil. ^{1}H-NMR (CDCl$_3$): δ 0.80 (t, 6H), 1.20-1.75 (6H), 1.90 (m, 1H), 2.15 (s, 12H), 2.35-2.60 (s, 24H), 2.65-3.40 (15H), 6.85 (b, 8H); ^{13}C-NMR (CDCl$_3$): δ 12.38, 20.71, 22.52, 22.66, 24.72, 27.55, 28.04, 39.19, 39.71,

15 41.02, 42.33, 42.62, 43.37, 48.81, 61.44, 131.76, 131.88, 133.10, 133.89, 138.66, 139.93, 142.17, 142.33, 142.57. MS-FAB (m/z) 1012 (M$^+$), 828 (M$^+$ - 184).

Compound 56 was obtained following the procedure described for compound **43**. From 1.26 g (1.24 mmol) of tetramide **55**, 300 mg (56%) of the tetrahydrochloride **56** was obtained; mp > 270°C (decomp). ^{1}H-NMR (D$_2$O): δ 1.35 (t, 6H), 1.60 (m, 1H), 1.80 (b,

20 3H), 2.15 (b, 6H), 2.50 (b, 1H), 3.20 (m, 13H), 3.45 (m, 2H); ^{13}C-NMR (D$_2$O): δ 13.23, 25.48, 25.73, 25.79, 31.69, 31.99, 43.40, 45.91, 46.43, 46.71, 48.07, 53.20, 75.28. MS-MALDI (m/z) 285 (M$^+$ + 1).

Compound 57: NaH (80%, 150 mg, 5 mmol) and NaBr (2.5 g, 25 mmol) were added to a solution of compound **52** (2.35 g, 4.9 mmol) in DMF (15 ml). The mixture was stirred

25 at 20°C for 30 min and a solution of 1-bromoethane (2.2 g, 25 mmol) in DMF (10 ml) was added in small portions. The mixture was stirred at 90°C for 3 h. The solvent was distilled off, the residue taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na$_2$SO$_4$) and evaporated. The product was purified by silica gel chromatography (hexane/ethyl acetate 9:1). The oily residue (1.5 g, 79%) crystallized

30 on standing; mp 68-69°C. ^{1}H-NMR (CDCl$_3$): δ 1.10 (t, 3H), 1.30-2.10 (6H), 2.25 (b,

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4H), 2.60 (s, 6H), 3.20 (m, 2H), 3.35 (m, 2H), 3.60 (m, 2H), 6.95 (s, 2H); ^{13}C-NMR (CDCl$_3$): δ 16.35, 20.93, 21.79, 22.89, 29.32, 29.37, 36.54, 38.12, 44.13, 61.40, 131.99, 132.80, 140.20, 142.52. MS-FAB 389 (M$^+$ + 1), 308 (M$^+$ - 80).

Compound 59 was obtained following the procedure described for compound **42**. From 700 mg (1.80 mmol) of compound **57** and 394 mg (0.9 mmol) of diamide **58**, 400 mg (37%) of tetramide **59** were obtained. ^{1}H-NMR (CDCl$_3$): δ 0.90 (t,6H), 1.25-1.80 (m,8H), 1.80-2.10 (m,8H), 2.15 (s, 12H), 2.40, 2.50 (s, 24H), 2.60-3.35 (m,6H), 2.85, 2.90 (s, 8H); ^{13}C-NMR (CDCl$_3$): δ 16.14, 20.85, 21.95, 21.99, 22.55, 25.49, 28.78, 28.88, 31.49, 37.87, 40.50, 40.83, 43.85, 44.06, 49.30, 61.42, 131.86, 131.96, 133.09, 133.40, 139.93, 139.98, 142.27, 142.40. MS-FAB (m/z) 1052 (M$^\oplus$), 891 (M$^+$ - 184).

Compound 60 was obtained following the procedure described for compound **43**. From 400 mg (0.38 mmol) of tetramide **59,** 95 mg (53%) of the tetrahydrochloride derivative were obtained; mp >270°C (decomp.) ^{1}H-NMR (D$_2$O): δ 1.30 (t, 6H), 1.60 (m, 2H), 1.80 (m, 6H), 1.95-2.35 (6H), 2.45 (m, 2H), 3.20 (m, 10H), 3.40 (m, 4H); ^{13}C-NMR (D$_2$O): δ 13.59, 25.34, 25.71, 31.75, 32.00, 43.34, 44.83, 48.02, 53.24, 64.52. MS-MALDI (m/z) 325 (M$^+$ + 1).

Compound 62: Mesitylenesulfonylchloride (3.27 g, 15 mmol) in dioxane (20 ml) was added dropwise to a stirred solution of **61** (1.3 g, 10 mmol) in dioxane (20 ml) and 50% KOH (15 ml) at 0°C. When addition was completed, the mixture was left over night at 20°C. Excess water was added, the solution cooled and the precipitate filtered off. Crystallization from ethylacetate - hexane gave compound **62** (2 g, 80%); mp 115-116°C. ^{1}H-NMR (CDCl$_3$): δ 2.35 (s, 3H), 2.55 (t, 2H), 2.65 (s, 6H), 3.25 (q, 2H), 5.15 (t, 1H), 7.0 (s, 2H); ^{13}C-NMR (CDCl$_3$): δ 19.07, 20.82, 22.78, 38.37, 117.56, 132.07, 133.0, 138.99, 142.67. MS-EI (m/z) 252 (M$^+$).

Compound 63: NaH (80%, 330 mg, 11 mmol) was added to a solution of compound **62** (2.52 g, 10 mmol) in DMF (20 ml) under N$_2$. The mixture was stirred for 30 min and a solution of compound **53** (3.82 g, 11 mmol) in DMF (10 ml) was added in small portions. The mixture was stirred at 70°C for 2h. The solvent was distilled off, the residue taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na$_2$SO$_4$) and evaporated to dryness. The product was purified by silica-gel

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chromatography (hexane - ethyl acetate 8:2). The oily residue (3.0 g, 57%) crystallized on standing; mp 105-106°C. ^{1}H-NMR (CDCl$_3$): δ 1.00 (t, 3H), 1.75 (m, 2H), 2.35 (s, 6H), 2.60 (14H), 3.10 (m, 6H), 3.45 (t, 3H), 6.90, 6.95 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 12.63, 16.94, 20.89, 22.67, 25.73, 40.27, 42.19, 42.51, 44.72, 117.36, 131.95, 132.22,

5   140.06, 140.34, 142.52, 143.33. MS-EI (m/z) 519 (M$^+$), 429 (M$^+$ - HCN).

Compound 65: The nitrile **63** (3.0 g, 5.7 mmol) was dissolved in a mixture of ethanol (150 ml) and concentrated hydrochloric acid (1.5 ml). PtO$_2$ was added (300 mg), the mixture was hydrogenated at 50 psi over night, the catalyst was filtered off and the solvent evaporated to afford an oily residue of compound **64**, which was used in the next

10   step without further purification. Free base ^{1}H-NMR (CDCl$_3$): δ 1.00 (t, 3H), 1.55 (m, 2H), 1.75 (m, 2H), 2.30 (s, 6H), 2.55 (14 H), 2.90-3.30 (8H), 6.95 (s, 4H); ^{13}C-NMR (CDCl$_3$): δ 12.64, 20.87, 22.69, 25.35, 30.93, 39.04, 40.12, 42.65, 43.11, 131.86, 133.10, 140.04, 142.43. MS-FAB (m/z) 524 (M$^+$ + 1).

Mesitylenesulfonylchloride (1.86 g, 8.55 mmol) in dioxane (15 ml) was added dropwise

15   to a stirred mixture of **64** (3.0 g, 5.7 mmol) dissolved in dioxane (30 ml) and 50% KOH (15 ml) at 0°C. The reaction mixture was allowed to reach room temperature and was kept for further 2 h. An excess of water was added and the mixture was extracted with chloroform, dried (Na$_2$SO$_4$) and evaporated to dryness. Purification was achieved by silica gel column chromatography using hexane - ethyl acetate (8:2) as eluant; 2.79 g

20   (69%) of **65** were obtained. ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 3H), 1.60 (m, 4H), 2.30 (s, 9H), 2.50 (s, 12H), 2.65 (s, 6H), 2.85 (m, 2H), 3.05 (6H), 3.20 (t, 2H), 5.00 (t, 1H), 6.95 (6H); ^{13}C-NMR (CDCl$_3$): δ 12.45, 20.81, 22.73, 25.23, 27.46, 39.19, 33.99, 42.49, 42.92, 43.17, 131.84, 133.05, 133.82, 138.80, 139.90, 141.92, 142.36, 142.64. MS-FAB (m/z) 705 (M$^{⊕}$).

25   **Compound 66** was obtained following the procedure described for compound **42**. From 705 mg (1 mmol) of **65** and 426 mg (1.1 mmol) of **57**, 470 mg (46%) of tetramide **66** was obtained as a glassy product. ^{1}H-NMR (CDCl$_3$): δ 0.85-1.10 (t, 6H), 1.35-2.10 (m,11H), 2.30 (s, 12H), 2.40-2.65 (m,24H), 2.75-3.55 (m,13H), 6.95 (m,8H); ^{13}C-NMR (CDCl$_3$): δ 12.64, 16.11, 20.91, 22.08, 22.75, 24.81, 25.09, 28.83, 29.07, 37.93, 40.08, 40.84,

30   42.50, 42.81, 43.11, 43.42, 49.11, 61.43. MS-FAB (m/z) 1013 (M$^+$ + 1).

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Compound **67** was obtained following the procedure described for compound **43**. From 470 mg (0.46 mmol) of tetramide **66**, 142 mg (71%) of the tetrahydrochloride derivative was obtained; mp >250 °C (decomp). ^{1}H-NMR (D$_2$O): δ 1.30 (t, 6H), 1.60 (m, 1H), 1.85 (b,s, 3H), 2.15 (m, 6H), 2.45 (m, 1H), 3.15 (m, 13H), 3.45 (m, 2H); ^{13}C-NMR (D$_2$O): δ

5   13.29, 13.57, 25.34, 25.44, 25.64, 31.68, 31.94, 43.27, 44.80, 45.86, 46.62, 47.42, 47.97, 53.19, 64.50. MS-MALDI 285 (M$^+$ + 1), 286 (M$^+$ + 2).

Compound 68a: 4-Cyanobenzaldehyde (Aldrich, 1.31 g, 10 mmol) was dissolved in 30 ml anhydrous MeOH followed by the addition of MgSO$_4$ (anhydrous, 1.5 g) and 1,4-diaminobutane (Aldrich, 0.44 g, 5 mmol) and the mixture was stirred under argon over

10   night. The suspension was cooled in an ice bath and NaBH$_4$ (2.0 g) was added in portions and stirring continued for 2 h at 0°C. The methanol was evaporated under vacuum and the resulting solid was partitioned between 35 ml H$_2$O and 50 ml CHCl$_3$. Some of the solid was not soluble in either the H$_2$O or the CHCl$_3$ and was filtered off and the aqueous layer was extracted with CHCl$_3$ (2 x 25 ml). The pooled organic layers were

15   dried (MgSO$_4$), evaporated and the solid was recrystallized from ethyl acetate - hexane, yield 1.1 g (35%); mp 90.6-90.8°C. ^{1}H-NMR (CDCl$_3$): δ 1.43 (broad, 2H, NH), 1.55 (m, 4H, CH$_2$), 2.63 (m, 4H, NCH$_2$), 3.85 (s, 4H, benzylic CH$_2$), 7.44 (m, 4H, Ph), 7.60 (m, 4H, Ph); ^{13}C-NMR (CDCl$_3$): δ 27.78, 49.28, 53.44, 110.65, 118.88, 128.52, 132.12, 146.21. MS (m/z) 318 (M$^+$), 185, 145, 131, 116 (100%), 70.

20   **Compound 68b** was prepared from 4-cyano-benzaldehyde and 1,5-diaminopentane as described above for **68a**; 42% yield; mp 92.9-93.0°C. ^{1}H-NMR (CDCl$_3$): δ 1.40 (m, 4H, NH, CH$_2$), 1.50 (m, 4H, CH$_2$), 2.59 (m, 4H, NCH$_2$), 3.83 (s, 4H, benzylic CH$_2$), 7.45 (m, 4H, Ph), 7.59 (m, 4H, Ph); ^{13}C-NMR (CDCl$_3$): δ 24.86, 29.87, 49.29, 53.40, 110.50, 118.85, 128.48, 132.04, 146.19. MS (m/z) 332 (M$^+$), 216, 199, 145, 116 (100%), 84.

25   **Compound 68c** was prepared from 4-cyanobenzyldehyde and 1,6-diaminohexane as described above for **68a**; 45% yield; mp 95.6-95.8°C. ^{1}H-NMR (CDCl$_3$): δ 1.35 (m, 4H, CH$_2$), 1.50 (m, 6H, NH, CH$_2$), 2.60 (t, J=6.92 Hz, 4H, NCH$_2$), 3.84 (s, 4H, benzylic CH$_2$), 7.44 (m, 4H, Ph), 7.60 (m, 4H, Ph); ^{13}C-NMR (CDCl$_3$): δ 27.17, 30.02, 49.42, 53.50, 110.65, 118.92, 128.55, 132.14, 146.27. MS (m/z) 346 (M$^+$), 230, 213, 145, 116

30   (100%) 98.

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Compound 69a: Dinitrile **68a** (0.75 g, 2.36 mmol) was dissolved in anhydrous THF, lithium bis(trimethylsilyl)amide (9.43 ml of a 1 m solution in THF) was added slowly under argon atmosphere. The mixture was stirred at room temperature for 2 h; then cooled in an ice bath, followed by the addition of 4 equivalents of 6N HCl in ether. A white solid precipitated immediately and was filtered after 12 h. The solid was recrystallized from ethanol-ether to afford 1.19 g of compound **69a** (93%). ^{1}H-NMR (D$_2$O): δ 1.87 (m, 4H, CH$_2$), 3.22 (m, 4H, CH$_2$N), 4.40 (s, 4H, benzylic CH$_2$), 7.74 (m, 4H, Ph), 7.91 (m, 4H, Ph); ^{13}C-NMR (DMSO-d$_6$): δ 22.68, 46.09, 49.28, 128.10, 128.47, 130.69, 138.15, 165.44. MS-ESI (m/z) 353.2 (M$^+$), 177.2 (100%).

Compound 69b was prepared from **68b** in 92% yield as described above for **69a**. ^{1}H-NMR (D$_2$O): δ 1.52 (m, 2H, CH$_2$), 1.80 (m, 4H, CH$_2$), 3.19 (m, 4H, NCH$_2$), 4.40 (s, 4H, benzylic CH$_2$), 7.75 (m, 4H, Ph), 7.91 (m, 4H, Ph); ^{13}C-NMR (DMSO-d$_6$): δ 24.90, 26.91, 48.96, 51.88, 130.29, 130.46, 132.43, 139.51, 167.52. MS-ESI (m/z) 367.2 (M$^+$), 350.2 (100%), 301.2.

Compound 69c was prepared from **68c** as described above for **69a** in 96% yield. ^{1}H-NMR (D$_2$O): δ 1.46 (m, 4H, CH$_2$), 1.78 (m, 4H, CH$_2$), 3.16 (m, 4H, NCH$_2$), 4.39 (s, 4H, benzylic CH$_2$), 7.74 (m, 4H, Ph), 7.91 (m, 4H, Ph); ^{13}C-NMR (DMSO-d$_6$): δ 25.24, 25.82, 46.73, 49.44, 128.35, 128.56, 130.81, 138.38, 165.58. MS-ESI (m/z) 381.2 (M$^+$), 191.2 (100%), 150, 116.

Compound 70: Triamide **18** (4.3 g, 5.8 mmol) was dissolved in 30 ml of DMF and 80% NaH (208 mg, 6.9 mmol) was added. The mixture was stirred under a N$_2$ atmosphere for 1 h and 1.12 g (7.5 mmol) of bromobutyronitrile dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 3 h at 90°C. The solvent was distilled-off and the residue was dissolved in chloroform and washed twice with a saturated solution of amonium chloride; dried (NaSO$_4$) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (6:4) as eluant gave the yellow oil **70** (3.7 g, 77%). ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 3H), 1.35 (m, 8H), 1.85 (m, 2H), 2.20 (t, 2H), 2.30 (s, 9H), 2.55 (s, 18H), 3.10 (m, 10H), 3.25 (t, 2H), 6.95 (s, 6H). MS-FAB (m/z) 823 (M$^+$ + Na), 639, 457.

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Compound 71: Nitrile **70** (3.7 g, 4.6 mmol) was dissolved in 20 ml of chloroform and 150 ml of ethanol were added. The mixture was reduced over 0.35 g of PtO_2 at 50 psi over night. The catalyst was filtered-off and the solvent evaporated to dryness. The oily residue was dried in vacuo for 2 h and dissolved in 50 ml of Cl_3CH and 12 ml 2N NaOH.

5   The mixture was cooled in an icewater bath with efficient magnetic stirring and 1.50 g (6.9 mmol) of mesitylene chloride dissolved in 10 ml of chloroform were added all at once. After 2 h the organic layer was separated, washed twice with a saturated solution of amonium chloride, dried ($NaSO_4$) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (7:3) as eluant provided the tetramide **71** as a

10   colorless oil (3.3 g, 73% over two steps). ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H), 1.40 (m, 12H), 2.30 (s, 12H), 2.60 (s, 24H), 2.80 (b, 2H), 3.10 (m, 12H), 4.70 (b, 1H), 6.90 (s, 8H). MS-FAB (m/z) 1010 ($M^+ + 1 + Na$), 826, 643.

Compound 72: The tetramide **71** (6.28 g, 6.3 mmol) was dissolved in 40 ml of DMF and 80% NaH (230 mg, 7.6 mmol) was added. The mixture was stirred under a N_2

15   atmosphere for 1 h and 1.30 g (8.8 mmol) of bromobutyronitrile dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 3h at 90°C, the solvent was distilled-off and the residue was extracted into chloroform and washed twice with a saturated solution of amonium chloride; dried ($NaSO_4$) and evaporated to dryness. Flash chromatography of the residue with hexane – ethyl acetate (7:3) as eluant provided the

20   nitrile **72** (5.0 g, 74%). ^{1}H-NMR ($CDCl_3$): δ 0.95 (t, 3H), 1.35 (m, 12H), 1.80 (m, 2H), 2.25 (t, 2H), 2.35 (s, 12H), 2.70 (s, 24H), 3.10 (m, 14H), 3.25 (t, 2H), 7.0 (s, 8H). MS-FAB (m/z) 1077 ($M^+ + 1 + Na$), 893, 711,586.

Compound 73: Nitrile **72** (6.0 g, 5.6 mmol) was dissolved in 20 ml of chloroform and 150 ml of ethanol were added. The mixture was reduced over 600 mg of PtO_2 at 50 psi

25   overnight. The catalyst was filtered-off and the solvent evaporated to dryness. The oily residue was dried in vacuo for 2 h and dissolved in 100 ml of chloroform and 15 ml 2N NaOH. The mixture was cooled in an icewater bath with efficient magnetic stirring, and 1.80 g (8.4 mmol) of mesitylene chloride dissolved in 10 ml of Cl_3CH was added all at once. After 2h the organic layer was separated, washed twice with a saturated solution of

30   amonium chloride, dried (Na_2SO_4) and evaporated to dryness. Flash chromatography of

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the residue using hexane – ethyl acetate (7:3) as eluant gave the pentamide **73** as a colorless oil (5.0 g, 71% over two steps). ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 3H), 1.35 (m, 16H), 2.30 (s, 15H), 2.55 (s, 30H), 2.75 (bs, 2H), 3.05 (m, 16H), 4.70 (b, 1H), 6.90 (s, 10H). MS-FAB (m/z) 1261 (M$^+$ - 1 + Na), 1077, 895.

5   **Compound 74**: Pentamide **73** (3.4 g, 2.7 mmol) was dissolved in 30 ml of DMF and 60% NaH (162 mg, 4.05 mmol) was added. The mixture was stirred under a N$_2$ atmosphere for 0.5 h and 2.3 g (10.8 mmol) of 2-bromoethanol benzylether dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 2 h at 80°C, the solvent was distilled-off and the residue was dissolved in chloroform and washed twice

10   with a saturated solution of amonium chloride, dried (NaSO$_4$) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (7:3) as eluant provided the product **74** (2.6 g, 70%). ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 3H), 1.30 (m, 16H), 2.30 (s, 15H), 2.50 (s, 30H), 2.90-3.20 (m, 18H), 3.25 (t, 2H), 2.35 (t, 2H), 4.35 (s, 2H), 6.95 (s, 10H), 7.20-7.35 (m, 5H). ^{13}C NMR (CDCl$_3$): δ 12.65, 20.84, 22.67, 22.71, 24.41, 24.66,

15   39.97, 44.48, 44.88, 46.59, 68.01, 72.95, 127.46, 127.57, 128.25, 131.83, 131.89, 133.28, 139.88, 139.95, 140.04, 142.16, 142.23. MS-FAB (m/z) 1394 (M$^+$ - 2 + Na) 1030.

Compound 75: Pentamide **74** (1.2 g, 0.87 mmol) was dissolved in 12 ml of methylene chloride followed by the addition of 30% HBr/acetic acid (16 ml) and phenol (3.0 g, 32 mmol). The mixture was stirred at room temperature overnight, water (16 ml) was

20   added, followed by extraction with methylene chloride (3 × 10 ml). The aqueous layer was evaporated in vacuo. The residue was dissolved in 2N NaOH (4 ml) and 50% KOH (4 ml) followed by extraction with chloroform (4 × 10 ml). After removal of the solvent the residue was dissolved in ethanol (20 ml) and acidified with concentrated hydrochloric acid (0.5 ml). The white precipitate (**75**) was recrystallized from aqueous ethanol (440

25   mg, 90%); mp above 270°C (decomp). ^{1}H-NMR (D$_2$O): δ 1.30 (t, 3H), 1.75 (b, 16H), 2.90-3.30 (m, 20H), 2.85 (t, 2H). ^{13}C NMR (D$_2$O): δ 13.29, 25.48, 25.59, 45.70, 49.04, 49.49, 49.67, 51.88, 59.39. MS-MALDI (m/z) 374 (M$^+$ + 1).

Compound 76: Pentamide **73** (850 mg, 0.68 mmol) was dissovled in DMF (15 ml) and 80% NaH (30 mg, 1 mmol) was added. The mixture was stirred under a N$_2$ atmosphere

30   at room temperature for 0.5 h and 137 mg (0.30 mmol) of **73** dissolved in 5 ml of DMF

were slowly added. The reaction mixture was heated for 2 h at 80°C, the solvent was distilled-off and the residue was dissolved in chloroform and washed twice with a saturated solution of amonium chloride, dried (NaSO$_4$) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate – methanol (6:4:0.1) as eluant afforded the product **76** (590 mg, 77%). ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 6H), 1.15-1.40 (m, 32H), 2.30 (s, 30H), 2.55 (s, 60H), 2.90-3.25 (m, 36H), 3.60 (d, 4H), 5.40 (t, 2H), 6.95 (s, 20H). MS-FAB 2553 (M$^+$ + Na).

Compound 77 was obtained following the procedure described for compound **75**. From 650 mg (0.25 mmol) of decamide **76**, 225 mg (81%) of decahydrochloride **77** was obtained; mp > 270°C (decomp). ^{1}H-NMR (D$_2$O): δ 1.30 (t, 6H), 1.75 (b, 32H), 3.10 (b, 36H), 3.75 (b, 4H), 6.05 (b, 2H); ^{13}C NMR (D$_2$O): δ 13.28, 25.57, 45.66, 49.00, 49.13, 49.64, 50.86, 131.15. MS-ESI 711 (M$^+$ + 1).

Compound 78 was obtained following the procedure described for compound **76**. From 850 mg of **73**, 360 mg (47%) of decamide **78** were obtained. ^{1}H-NMR (CDCl$_3$): δ 0.95 (t, 6H), 1.15-1.45 (m, 32H), 2.30 (s, 30H), 2.55 (s, 60H), 2.90-3.20 (b, 36H), 3.65 (d, 4H), 5.40 (t, 2H), 6.90 (s, 20H). MS-FAB (m/z) 2553 (M$^+$ + Na).

Compound 79 was obtained following the procedure described for compound **75**. From 330 mg (0.13 mmol) of decamide **78**, 127 mg (90%) of decahydrochloride **79** was obtained; mp > 270°C (decomp). ^{1}H-NMR (D$_2$O): δ 1.30 (t, 6H), 1.80 (b, s, 32H), 3.10 (b, 36H), 3.85 (d, 4H), 6.0 (t, 2H). ^{13}C NMR (D$_2$O): δ 13.31, 25.59, 45.71, 46.83, 49.05, 49.39, 49.69, 129.21. MS-ESI (m/z) 512 (M$^+$ + 2).

Compound 96: A suspension of NaH (60% in mineral oil, 336 mg, 14 mmol) in DMF (50 ml) was slowly added to a stirred solution of benzyl-4-bromobuty ether (3.645 g, 15 mmol) and mesitylenesulfonamide **18** (7.33g, 10 mmol) in DMF (180 ml) at 0^{0}C. The reaction mixture was stirred for 10 h at 50^{0}C, quenched with 15 ml of H$_2$O at 0^{0}C, acidified to pH=7 with 5% HCl, extracted with Et$_2$O, washed with brine, dried (Na$_2$SO$_4$), and purified on a column (SiO$_2$, EtOAc/Hexane = 3:7); yield 6.9 g, (77%). ^{1}H-NMR (CDCl$_3$): δ 0.98 (t, J=7.1 Hz, 3H, CH$_3$), 1.25-1.30 (m, 12H, CH$_2$), 2.27 (s, 3H, CH$_3$), 2.29 (s, 6H, CH$_3$), 2.55 (s, 18H, CH$_3$), 3.0-3.2 (m, 12H, CH$_2$), 3.31 (t, J=6.0 Hz, CH$_2$), 4.41 (s, 2H, CH$_2$), 6.91 (s, 2H, Ph), 6.92 (s, 4H, Ph), 7.2-7.4 (m, 5H, Ph).

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Compound 97: A solution of HBr 30% in AcOH (45 ml) was added to a stirred solution of **96** (2 g, 2.24 mmol) and phenole (6.324 g, 67.2 mmol) in CH_2Cl_2 (23 ml) at 0^0C. The cooling bath was removed and the reaction mixture was stirred for 24 h at 20^0 C. The reaction mixture was quenched with H_2O (45 ml), washed with CH_2Cl_2, and concentrated to dryness *in vacuo*. The residue was cooled to 0^0 C, basified with 2N NaOH (5 ml), followed by 50% KOH (5 ml). The product was extracted with $CHCl_3$ (7 x 10 ml); yield 475 mg (81%). ^{1}H-NMR (D_2O): δ 1.10 (t, J=7 Hz, 3H, CH_3), 1.45-1.70 (m, 12H, CH_2), 2.55-2.70 (m, 12H, CH_2), 3.57 (t, J=5.0 Hz, CH_2).

Compound 98: A solution of Na_2CO_3 (10%, 26 ml) was added to triamine **97** (830 mg, 3.20 mmol) in dioxane (21 ml). Di-*tert*-butyl dicarbonate (3.25 g, 24 mmol) solution in dioxane (21 ml) was added into the reaction mixture at 0^0 and stirred for 10 h at 20^0 C. The reaction mixture was diluted with $CHCl_3$ (200 ml), washed with H_2O, brine, dried (Na_2SO_4), concentrated and purified on a column (SiO_2, EtOAc/hexane=4:6); yield 1.7 g, (96%). ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7.1 Hz, 3H, CH_3), 1.45-1.65 (m, 39H, CH_2, CH_3), 3.1-3.3 (m, 12H, CH_2), 3.67 (t, J=6 Hz, CH_2).

Compound 99: Diethyl azodicarboxylate (196 mg, 1.13 mmol) in THF (0.6 ml) was added to a cold mixture of **98** (630 mg, 1.127 mmol), triphenylphosphine (296 mg, 1.13 mmol) and phthalimide (166 mg, 1.13 mmol) in THF (1.2 ml), stirred for 10 h, concentrated *in vacuo*, and purified on a column (SiO_2, EtOAc/hexane=3:7); yield 835 mg (97%). ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7, 3H, CH_3), 1.35-1.80 (m, 39H, CH_2, CH_3), 3.1-3.35 (m, 12H, CH_2), 3.70 (t, J=6.7 Hz, 2H, CH_2), 7.68-7.80 (m, 2H, Phth), 7.80-7.87 (m, 2H, Phth).

Compound 100: A mixture of **99** (275 mg, 0.4 mmol), hydrazine monohydrate (98%, 43.5 μl, 0.85 mmol) in EtOH were heated at 80^0 C for 45 min. The precipitate was filtered and washed with cold EtOH, filtrate was combined with washings, concentrated and dried *in vacuo*; yield 220 mg (99%). ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7.0 Hz, 3H, CH_3), 1.25-1.57 (m 39H, CH_2, CH_3), 2.71 (t, J=6.7 Hz, 2H, CH_2), 3.1-3.3 (m, 12H, CH_2).

Compound 101 Mesoporphyrin IX dihydrochloride (70 mg, 0.11 mmol) was dissolved in DMF (1 ml), combined with HBTU (83 mg, 0.22 mmol) and diisopropylethyl amine (174 μl, 1 mmol). The mixture was stirred for 5 min, combined with **100** (167 mg, 0.3

mmol), stirred for 10 h, quenched with H_2O, concentrated *in vacuo*, dissolved in $CHCl_3$, washed with H_2O (5 times), 2% $KHSO_4$, $NaHCO_3$ solution, dried (Na_2SO_4), and purified by a column chromatography (SiO_2, $MeOH/CHCl_3 = 1:20$); yield 177 mg (98%). ^{1}H-NMR ($CDCl_3$): δ 1.06 (t, J=6.9, 6H, CH_3), 1.1-1.5 (m, 78H, CH_2, CH_3), 1.86 (t, J=6.6 Hz, 6H, CH_3), 2.9-3.25 (m, 28H, CH_2), 3.62 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 4.07 (q, J=7.5 Hz, 4H, CH_2), 4.43 (t, J=7Hz, 4H, CH_2), 10.07 (s, 3H, CH), 10.27 (s, 1H).

Compound 102: Trifluoroacetate (1 ml) was added to a solution of **101** (165 mg, 0.1 mmol) in CH_2Cl_2 (2 ml) at 0^0 C, stirred for 1.5 h, and concentrated *in vacuo* at 20^0 C. The product was dried *in vacuo* for 3h, dissolved in 10 ml of 10% HCl, washed with $CHCl_3$, and the aqueous part was concentrated and dried *in vacuo* at 20^0 C; yield 100 mg (75%), purple solid, mp >200^0 C. Purity 96% (HPLC). ^{1}H-NMR (D_2O): δ 0.7-1.0 (m), 1.0-1.2 (m), 1.29 (t, J=7.3 Hz), 1.4-1.7 (m), 2.6-2.35 (m), 2.72 (t, J=7.2 Hz), 2.9-3.2 (m), 3.6-3.75 (m), 3.76 (s), 3.79 (s), 4.29 (q, J=7.6 Hz), 4.4-4.7 (m). MS-MALDI (m/z): 1193 (M^+ + 4 HCl), 1156 (M^+ + 3 HCl), 1119 (M^+ + 2 HCl), 1083 (M^+ + HCl), 1048 (M^+ + 1).

Compound 103 was prepared from **71** in 85% yield as described above for **96**. ^{1}H-NMR ($CDCl_3$): δ 0.97 (t, J=7 Hz, 3H, CH_3), 1.3-1.55 (m, 16H, CH_2), 2.27 (s, 3H, CH_3), 2.29 (s, 9H, CH_3), 2.55 (s, 24H, CH_3), 3.0-3.25 (m, 16H, CH_2), 3.31 (t, J=5.8 Hz, 2H, CH_2), 4.41 (s, 2H, CH_2), 6.90 (s, 2H, Ph), 6.92 (s, 6H, Ph).

Compound 104 was prepared from **103** in 80% yield as described for **97**. ^{1}H-NMR ($CDCl_3$): δ 1.10 (t, J=7.2 Hz, 3H, CH_3), 1.5-1.8 (m, 16H, CH_2), 2.5-2.75 (m, 16H, CH_2), 3.57 (t, J=5.5 Hz, 2H, CH_2). ^{13}C-NMR ($CDCl_3$): δ 15.23, 27.55, 27.92, 28.58, 32.35, 44.02, 49.35, 49.66, 49.80, 62.32.

Compound 105 was prepared from **104** in 98% yield as described for **98**. ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7.1 Hz, 3H, CH_3), 1.4-1.7 (m, 52 H, CH_2, CH_3), 3.05-3.3 (m, 16H, CH_2), 3.67 (t, J=5.8 Hz, 2H, CH_2).

Compound 106: Oxalyl chloride (2N solution in CH_2Cl_2, 0.821 µl, 1.642 mmol) was diluted with anhydrous CH_2Cl_2 (6 ml) at -60^0 C. DMSO (223 µl, 2.59 mmol) in CH_2Cl_2 (3 ml) was added to the mixture, the latter stirred for 5 min at -60^0 C, and **105** (1.115 g,

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1.525 mmol) in CH_2Cl_2 (9 ml) was introduced into the reaction. After 30 min of stirring at -60^0 C triethylamine (1.06 ml, 14.46 mmol) was added into the reaction mixture and the temperature was allowed to rise to 20^0 C (ca. 1.5 h). The reaction mixture was diluted with CH_2Cl_2, washed with H_2O, $NaHCO_3$, and brine. The product was concentrated to dryness *in vacuo* and purified by column chromatography (SiO_2, EtOAc/Hexane= 3:7); yield 989 mg (89%). ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7.0 Hz, 3H, CH_3), 1.4-1.6 (m, 48H, CH_2, CH_3), 1.84 (m, 2H, CH_2), 2.45 (t, J=6.8, 2H, CH_2), 3.05-3.3 (m, 16H, CH_2), 9.78 (s, 1H, CHO).

Compound 107: Platinum oxide (100 mg) was reduced in methanol (30 ml) in the hydrogen atmosphere at 30 psi. for 15 min. Product **106** (989 mg, 1.36 mmol) was dissolved in a 2M solution of ethylamine in EtOH (7 ml), added to the hydrogenation flask, and hydrogenated for 10 h at 50 psi. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness *in vacuo*; yield 1.0 g (99%). ^{1}H-NMR ($CDCl_3$): δ 1.09 (t, J=7.6 Hz, 3H, CH_3), 1.12 (t, J=7.2 Hz, 3H, CH_3), 1.3-1.65 (m, 50H, CH_2, CH_3), 1.66 (m, 2H, CH_2), 2.71 (m, 2H, CH_2), 3.1-3.3 (m, 18H, CH_2). MS-MALDI (m/z):758.8 (M^+, 100%), 744 (30%).

Compound 108 was prepared from **107** in 98% yield as described above for **101**. ^{1}H-NMR ($CDCl_3$): δ 0.8-1.8 (m), 1.87 (t, J=7.3 Hz, 6H, CH_3), 2.5-3.5 (m), 3.65 (s, 6H, CH_3), 3.67 (s, 6H, CH_3), 4.10 (q, J=7.5, 4H, CH_2), 4.46 (t, J=6.8, 4H, CH_2), 10.11 (bs, 4H, porph. core).

Compound 109 was prepared from **108** in 75% yield as described above for **102**; purple solid, mp >200^0 C. Purity 96% (HPLC). ^{1}H-NMR (D_2O): δ 0.25-0.5 (m), 0.6-0.8 (m), 1.1-1.5 (m), 1.5-2.0 (m), 1.32 (t), 2.6-3.4 (m), 3.6-3.9 (m), 3.78 (s), 3.82 (s), 4.2-4.6 (m). MS-MALDI (m/z): 1246.22 (M^+ +1), 623.82 (M^{2+}).

Compound 111: Nitrile **70** (274 mg, 0.343 mmol) was hydrogenated in a 5% solution of $CHCl_3$ in EtOH (20 ml) in the presence of PtO_2 (60 mg) at 50 psi for 15 h. The reaction mixture was filtered through Celite and concentrated to dryness *in vacuo* to yield **110** (288 mg, 100%). Amine **110** was utilized without further purification. Mesoporphyrin IX dihydrocloride (100 mg, 0.156 mmol) was dissolved in DMF (4 ml), HBTU (118 mg,

0.312 mmol) and DIEA (174 μl, 2 mmol) were added to the solution, the latter stirred for

5 min, combined with **110,** and kept for 15 h. The reaction mixture was quenched with

0.5 ml of H_2O, concentrated *in vacuo*, dissolved in $CHCl_3$, washed with 3% HCl solution,

brine, dried (Na_2SO_4), and purified on a column (SiO_2, $CHCl_3$/MeOH=15:4); yield 327

5 mg (98%). ^{1}H NMR ($CDCl_3$): δ 0.5-0.8 (m), 0.8-1.1 (m), 0.87 (t, J=7 Hz), 1.1-1.3 (m),

1.5-1.8 (m), 1.88(q, J=5 Hz), 2.0-2.3 (m), 2.10 (s, CH_3-Mes), 2.13 (s, CH_3-Mes), 2.16 (s,

CH_3-Mes), 2.18 (s, CH_3-Mes), 2.43 (s, CH_3-Mes), 2.49 (s, CH_3-Mes), 2.7-2.9 (m), 2,9-3.2

(m), 3.64 (s, porph), 3.66 (s, porph), 4.1 (q, porph.), 4.4 (t, porph.), 6.69 (s, NH), 6.80 (s,

NH), 6.85 (s, NH), 6.93 (s, NH), 10.07 (s, porph), 10.09 (s, porph), 10.22 (s, porph.).

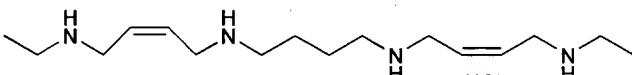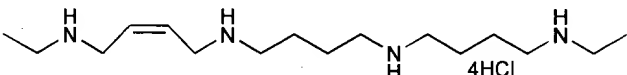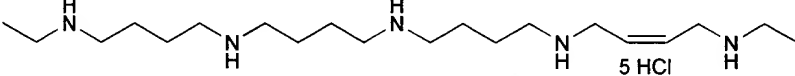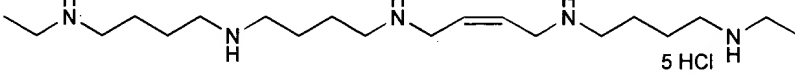10 MALDI (m/z): 2141.03 (M^+ +1), 1071.22 (M^{2+}).

Compound 112: Porphyrin **111** (350 mg, 0.163 mmol) was stirred in a suspension of

$LiAlH_4$ (12.4 mg, 0.326 mmol) in THF (1 ml) for 24 h at 50^0 C. The reaction mixture was

cooled to 0^0 C, quenched with H_2O (0.1 ml), basified with 2N NaOH (0.5 ml), then

diluted with CH_2Cl_2, filtered through Celite, washed with H_2O, dried (Na_2SO_4), and

15 concentrated *in vacuo*. The product was stirred in a mixture of phenol (920 mg, 9.4

mmol) in CH_2Cl_2 (8 ml), and 30% solution of HBr in AcOH (7 ml) for 15 h. The reaction

mixture was cooled to 0^0 C, diluted with 10 ml of H_2O and the aqueous layer was filtered

and washed with CH_2Cl_2. Following drying *in vacuo* of the aqueous solution, the residue

was basified with 2N NaOH (1 ml) and 16 N NaOH (1 ml) and the reaction product was

20 extracted with $CHCl_3$. The product **112** was purified by HPLC and converted to its

hydrochloride by dissolution in 10% HCl and evaporation of the acid *in vacuo*; purple

solid, mp 200^0 C. Purity 96% (HPLC). ^{1}H NMR (D_2O): δ 1.32 (t, J=7.3 Hz, 6H, CH_3),

1.25-1.35 (m, 30H, CH_2), 2.5-2.65 (m, 4H, CH_2), 3.0-3.3 (m, 32H, CH_2), 3.4-3.5 (m, 4H,

CH_2), 3.55-3.65 (m, 6H, CH_3), 3.73 (s, 3H, CH_3), 3.75 (s, 3H, CH_3), 4.0-4.25 (m, 4H,

25 CH_2), 4.3-4.5 (m, 4H). MALDI (m/z): 1073.8 (M^+ + NH_4Cl), 1020.0 (M^+ + 1), 510.62

(M^{2+}), 340.82 (M^{3+}).

Compound 113: Diisobutylaluminum hydrate (1.16 ml of 1.5 M solution in toluene, 1.74

mmol) was added into a solution of mesoporphyrin IX dimethyl ester (500 mg, 84 mmol)

in CH_2Cl_2 (10 ml) at -78^0 C, stirred at this temperature for 1h, quenched with saturated

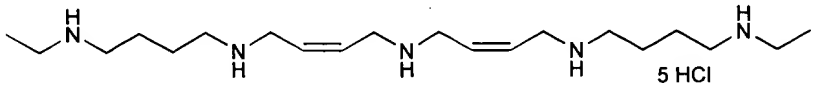30 solution of NH_4Cl (1 ml), followed by 3.7% solution of HCl (2 ml). The temperature of
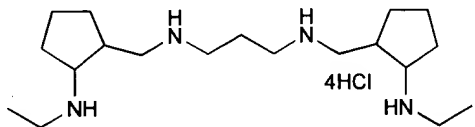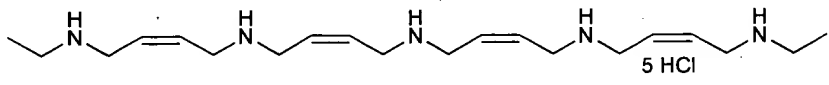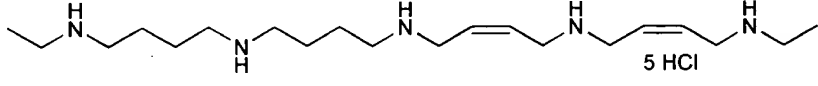
the reaction mixture was allowed to rise to 20^0C, the product was extracted with CH_2Cl_2, dried (Na_2SO_4), and purified on a column (SiO_2, EtOAc/Hexane=3:7), yield 330 mg (73%). ^{1}H-NMR ($CDCl_3$): δ 1.86 (t, J=7.6 Hz, 6H, CH_3), 3.39 (t, J=7.4 Hz, 6H, CH_3), 3.60 (s, 6H, CH_3), 3.62 (s, 6H, CH_3), 4.0-4.2 (m, 4H, CH_2), 4.25-4.45 (m, 4H, CH_2), 9.97

5   (s, 1H), 10.04 (s, 1H), 10.05 (s, 1H), 10.058 (s, 1H), 10.062 (s, 1H), 10.07 (s, 1H).

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Table 1. Polyamine analogs

Compound	Structure
SL-11090	H H [cyclobutane] H H structure (bis-propylamine substituted cyclobutane) •4HCl
SL-11091	Ethyl-NH-propyl-NH-CH2-C≡C-CH2-NH-propyl-NH-ethyl •4HCl
SL-11092	Methyl-NH-ethyl-NH-CH2-C≡C-CH2-NH-ethyl-NH-methyl •4HCl
SL-11101	Ethyl-NH-butyl-NH-CH2-CH=CH-CH2-NH-butyl-NH-ethyl • 4HCl
SL-11103	Ethyl-NH-butyl-NH-CH2-C≡C-CH2-NH-butyl-NH-ethyl •4HCl
SL-11114	Ethyl-NH-CH2-CH=CH-CH2-NH-butyl-NH-CH2-CH=CH-CH2-NH-ethyl 4HCl
SL-11118	Ethyl-NH-CH2-CH=CH-CH2-NH-butyl-NH-butyl-NH-ethyl 4HCl
SL-11121	Ethyl-NH-butyl-NH-butyl-NH-butyl-NH-CH2-CH=CH-CH2-NH-ethyl 5 HCl
SL-11122	Ethyl-NH-butyl-NH-butyl-NH-CH2-CH=CH-CH2-NH-butyl-NH-ethyl 5 HCl

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SL-11123	H H H H H ... N N N N N ... 5 HCl
SL-11124	H H ... N N ... 4HCl ... NH NH
SL-11126	H H H H H ... N N N N N ... 5 HCl
SL-11127	H H H H H ... N N N N N ... 5 HCl
SL-11128	H H H H H ... N N N N N ... 5 HCl
SL-11129	H H H H H ... N N N N N ... 5 HCl
SL-11130	H H H H H ... N N N N N ... 5 HCl
SL-11132	H H H ... N N N ... 4HCl ... NH
SL-11133	H H H H H ... N N N N N ... 5 HCl
SL-11134	H H ... H₂N N N NH₂ ... NH NH ... 4HCl

SL-11136	
SL-11137	
SL-11141	
SL-11144	
SL-11150	
SL-11201	

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SL-11202	10 HCl

Table 2. Polyamine-porphyrin conjugates

SL-11161	
SL-11162	
SL-11164	
SL-11177	
SL-11184	
SL-11202	

5

EXAMPLE 2

In vitro testing of the efficacy of novel polyamine analogs against tumor cell lines

5 These experiments are designed to evaluate the newly synthesized compounds described above against cultured human carcinoma cell lines for their effects on cell growth, cell-cycle regulation and polyamine regulatory responses. Additional methods for testing compounds are described in U.S. Patent No. 5,889,061.

As shown in Table 3 and Figs. 1-32, several novel conformationally restricted

10 polyamine analogs were tested for anti-proliferative properties against cancer cells. Table 3 illustrates the concentration in µM of the various novel polyamine analogs needed for 50% growth inhibition (ID_{50}) values for human cancer cell lines LNCaP, PC-3, DuPro (all three human prostate cancer cell lines), HT-29 (colon cancer cell line), A549 (lung cancer cell line), MCF7 (breast cancer cell line), and U251 MG-NCI (brain

15 cancer cell line). Figs. 1-32 show a representative plot of the effects of some of these novel analogs on the growth of human tumor cell lines, as determined by MTT (methyl thiazol tetrazolium) assay; known anti-proliferative polyamine analogs BE-333, BE-343, BE-444, and BE-4444 were used for comparative purposes.

Cell Lines and Media

20 Human breast cancer cell line MCF7 was grown in Richter's Improved Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS) and 2.2 g/L sodium bicarbonate. Human brain tumor cell line U251 MG-NCI was grown in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS. Human lung cancer cell line A549 was grown in Ham's F-12K medium (Cellgro, Mediatech, Inc., VA), supplemented

25 with 10% FBS and 2 mM L-glutamine. Human colon cancer cell line HT29 was cultured in McCoy's 5A medium (Gibco, BRL, Gaithersburg, MD) supplemented with 10% FBS. Human prostate cancer cell lines PC-3, LNCAP and DuPro were grown in RPMI 1640 Medium (Cellgro, Mediatech, Inc., VA) supplemented with 10% FBS. Another human prostate cancer cell line DU145 was grown in Dulbecco's Modified Eagle's Medium

30 (Gibco, BRL, Gaithersburg, MD) supplemented with 5% FBS. The A549, MCF7, PC3,

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LNCAP and DuPro cell lines were cultured in 100 units/mL penicillin and 100 µg/mL streptomycin. HT29 and U251MG cell lines were grown in 50 µg/mL gentamycin (Gibco, BRL, Gaithersburg, MD). DU145 cell line was maintained in 1% antibitic-antimycotic solution (Sigma, St. Louis, MO). The cell cultures were maintained at 37°C in 5%CO_2/95% humidified air. DuPro cells were obtained from M. Eileen Dolan, University of Chicago. All other cells are available from the American Type Culture Collection, Rockville, MD.

MTT assay

A conventional MTT assay was used to evaluate percent cell survival. Exponentially growing monolayer cells were plated in 96-well plates at a density of 500 cells per well and allowed to grow for 24 hours. Serial dilutions of the drugs were added to the wells. Six days after drug treatment, 25 µl of MTT solution (5 mg/ml) was added to each well and incubated for 4 hours at 37°C. Then 100 µl of lysis buffer (20% sodium dodecyl sulfate, 50% DMF, and 0.8% acetic acid, pH 4.7) was added to each well and incubated for an additional 22 hours. A microplate reader ("EMAX"-brand, Molecular Devices, Sunnyvale, Calif.) set at 570 nm was used to determine the optical density of the cultures. Results are expressed as a ratio of the optical density in drug-treated wells to the optical density in wells treated with vehicle only.

TABLE 3

	PC-3	DU-145	DUPRO	HT-29	A549	MCF7	U251MG
BE-4444	0.54	0.07	0.2	0.8	0.4	>31.25	NT
SL-11029	24.5	0.32	NT	>31.25	>31.25	>31.25	>31.25
SL-11090	>31.25	>31.25	NT	>31.25	>31.25	>31.25	>31.25
SL-11091	>31.25	1.33	NT	>31.25	>31.25	>31.25	>31.25
SL-11092	>31.25	1.7	NT	>31.25	>31.25	>31.25	>31.25
SL-11093	14.3	0.01	0.06	0.40	0.26	0.66	NT

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SL-11094	>31.25	12.6	NT	28.8	>31.25	>31.25	>31.25
SL-11098	1.4	0.018	0.08	0.40	0.51	>31.25	0.10
SL-11099	2.5	0.014	0.08	1.00	0.65	26.3	0.11
SL-11100	4.7	0.021	0.29	2.00	2.20	>31.25	0.22
SL-11101	7.7	0.218	0.85	5.20	0.15	>31.25	1.70
SL-11102	>31.25	0.027	0.15	0.73	12.40	>31.25	0.15
SL-11103	>31.25	2.8	NT	29.4	>31.25	>31.25	9.50
SL-11104	>31.25	9.4	NT	25.8	0.43	>31.25	14.71
SL-11105	>31.25	1.6	>31.25	25.2	>31.25	>31.25	25.9
SL-11108	2.2	0.13	0.98	2.00	>31.25	>31.25	2.00
SL-11114	0.70	0.135	0.64	3.6	>31.25	NT	NT
SL-11118	1.65	0.05	0.25	0.98	0.21	NT	NT
SL-11119	>31.25	0.08	0.44	0.97	NT	NT	NT
SL-11121	0.52	0.08	0.40	0.80	>31.25	17.0	NT
SL-11122	>31.25	0.80	0.56	0.80	>31.25	>31.25	NT
SL-11123	>31.25	0.51	>31.25	10.42	>31.25	>31.25	NT
SL-11124	>31.25	>31.25	>31.25	>31.25	>31.25	>31.25	NT
SL-11126	0.20	0.51	1.10	1.50	>31.25	0.70	NT
SL-11127	>31.25	0.22	1.3	2.91	NT	NT	NT
SL-11128	0.50	0.14	1.25	1.35	NT	NT	NT
SL-11129	1.70	0.32	NT	NT	NT	NT	NT
SL-11130	>31.25	0.43	NT	NT	NT	NT	NT

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NT indicates not tested.

5

EXAMPLE 3

In vivo testing of anti-tumor activity of polyamine analogs

Analogs found to have potent or mechanism-based anti-proliferative activity *in*

10   *vitro* towards cultured carcinoma cells are evaluated in *in vivo* model systems. The first

goal is to determine the relative toxicity of the analogs in non-tumor-bearing animals,

such as DBA/2 mice. Groups of three animals each are injected intraperitoneally with

increasing concentrations of an analog, beginning at 10 mg/kg. Toxicity, as indicated by

morbidity, is closely monitored over the first 24 hr. A well-characterized polyamine

15   analog, such as BE-333, is used as an internal standard in these studies, since a data base

has already been established regarding acute toxicity via a single dose treatment relative

to chronic toxicity via a daily x 5 d schedule. Thus, in the case of new analogs, single

dose toxicity relative to BE-333 is used to project the range of doses to be used on a daily

x 5 d schedule.

20   After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor

activity is determined. Tumors are subcutaneously implanted into nude athymic mice by

trocar and allowed to reach 100-200 mm^3 before initiating treatment by intraperitoneal

injection daily x 5 d. Analogs are given in a range between 10 and 200 mg/kg. Analogs

are evaluated at three treatment dosages with 10-15 animals per group (a minimum of

25   three from each are used for pharmacodynamic studies, described below). Mice are

monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is

determined by multi-directional measurement from which volume in mm^3 is calculated.

Tumors are followed until median tumor volume of each group reaches 1500 mm^3 (i.e.,

20% of body weight), at which time the animals are sacrificed. Although the initial anti-

30   tumor studies focused on a daily x 5 d schedule, constant infusion is performed via Alzet

pump delivery for 5 days since this schedule dramatically improves the anti-tumor

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activity of BE-333 against A549 human large cell lung carcinoma. Sharma et al. (1997) *Clin. Cancer Res.* 3:1239-1244. In addition to assessing anti-tumor activity, free analog levels in tumor and normal tissues are determined in test animals.

5

EXAMPLE 4

In vitro *testing of porphyrin-polyamine conjugates*

Using the protocols of Example 2, the porphyrin-polyamine conjugates were tested for activity *in vitro* in various cancer cell lines. The results are shown in Table 4 and Figs. 45-49.

10

Table 4

Effect of Polyamine Porphyrin Complexes on the Growth of Human Prostate Cell Lines by MTT Assay.

15

Compounds	IC_{50} (μM) in different cell lines			
	PC3	DUPRO	LNCAP	DU145
SL-11162	2.8	1.9	4.8	
SL-11177	0.9	0.7	4.8	
SL-11184	1.4	1.5		8.4
SL-11202	1.15	1.3		4.6

EXAMPLE 5

In vivo *testing of porphyrin-polyamine conjugates*

20   Conjugates found to have potent or mechanism-based anti-proliferative activity *in vitro* towards cultured carcinoma cells are evaluated in *in vivo* model systems. The first goal is to determine the relative toxicity of the conjugates in non-tumor-bearing animals, such as DBA/2 mice. Groups of three animals each are injected intraperitoneally with increasing concentrations of an conjugate, beginning at 10 mg/kg. Toxicity, as indicated

25   by morbidity, is closely monitored over the first 24 hr. A well-characterized compound, such as BE-333, is used as an internal standard in these studies, since a data base has

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already been established regarding acute toxicity via a single dose treatment relative to chronic toxicity via a daily x 5 d schedule. Thus, in the case of new conjugates, single dose toxicity relative to BE-333 is used to project the range of doses to be used on a daily x 5 d schedule.

5   After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor activity is determined. Tumors are subcutaneously implanted into nude athymic mice by trocar and allowed to reach 100-200 mm^3 before initiating treatment by intraperitoneal injection daily x 5 d. Conjugates are given in a range between 10 and 200 mg/kg. Conjugates are evaluated at three treatment dosages with 10-15 animals per group (a

10   minimum of three from each are used for pharmacodynamic studies, described below). Mice are monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is determined by multi-directional measurement from which volume in mm^3 is calculated. Tumors are followed until median tumor volume of each group reaches 1500 mm^3 (i.e., 20% of body weight), at which time the animals are sacrificed. Although

15   the initial anti-tumor studies focused on a daily x 5 d schedule, constant infusion is performed via Alzet pump delivery for 5 days since this schedule dramatically improves the anti-tumor activity of BE-333 against A549 human large cell lung carcinoma. Sharma et al. (1997) *Clin. Cancer Res.* 3:1239-1244. In addition to assessing anti-tumor activity, free conjugate levels in tumor and normal tissues are determined in test animals.

20

      Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those

25   skilled in the art that certain minor changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims. All references disclosed herein, including U.S. Patent No. 5,889,061, are hereby incorporated by reference in their entirety.